TECHNICAL NOTE

TruSight™ Oncology 500 High-Throughput on the NovaSeq™ X Series

Enable reliable comprehensive genomic profiling with increased throughput and flexibility

illumina®

High-throughput comprehensive genomic profiling

TruSight Oncology 500 High-Throughput is a next-generation sequencing (NGS)–based assay that simultaneously analyzes both DNA and RNA to enable in-house comprehensive genomic profiling (CGP). With an advanced enrichment chemistry that employs up to 192 10-bp unique dual indexes, TruSight Oncology 500 High-Throughput is ideally suited for scalable sequencing on high-throughput sequencing systems, such as the NovaSeq X and NovaSeq X Plus Systems (collectively known as the NovaSeq X Series). The NovaSeq X Series offers the throughput and accuracy needed to enable data-intensive applications and deliver meaningful insights at scale, while reducing the cost per gigabase (Gb) by up to 60% compared to the NovaSeq 6000 System.[1] The combination of TruSight Oncology 500 High-Throughput and the NovaSeq X Series enables CGP of all major variant classes plus gene signatures, including tumor mutational burden (TMB) and microsatellite instability (MSI), from formalin-fixed paraffin-embedded (FFPE) tissue in a single integrated workflow (Table 1, Figure 1).

This application note compares the performance of TruSight Oncology 500 High-Throughput on the NovaSeq X Series to performance on the NovaSeq 6000 System. Results demonstrate the same high-accuracy results achieved on the NovaSeq X Series, with the added benefits of reduced sequencing times and greater throughput.

Table 1: TruSight Oncology 500 High-Throughput performance on the NovaSeq 6000 System and NovaSeq X Series

System	NovaSeq 6000 System or NovaSeq 6000Dx Instrument (research mode)[a]	NovaSeq X Series[a]
Sample throughput	16–192 samples per run	Single flow cell: 32–480 samples per run Dual flow cell: 32–960 samples per run
Panel size	1.94 Mb DNA, 358 kb RNA	1.94 Mb DNA, 358 kb RNA
DNA input requirement	40 ng	40 ng
RNA input requirement	40–80 ng	40–80 ng
FFPE input requirement	Minimum recommendation of 2 mm^3 from FFPE tissue samples	Minimum recommendation of 2 mm^3 from FFPE tissue samples
Total assay time	4–5 days from nucleic acid to variant report	4–5 days from nucleic acid to variant report
Sequence run time	19 hr (SP and S1), 25 hr (S2), or 36 hr (S4)	18.5 hr (1.5B), 20 hr (10B), or 33 hr (25B)
Sequence run	2 × 101 cycles	2 × 101 cycles
Software version	DRAGEN TruSight Oncology v2.5.2+	DRAGEN TruSight Oncology v2.5.2+
Limit of detection	5% VAF for small variants 5 copies per ng RNA input for fusions (80 ng input) CNVs: 2.2× fold-change for amplifications 0.5× fold-change for deletions	5% VAF for small variants 5 copies per ng RNA input for fusions (80 ng input) CNVs: 2.2× fold-change for amplifications 0.5× fold-change for deletions
Analytical sensitivity	> 96% (for all variant types at 5% VAF)	> 96% (for all variant types at 5% VAF)
Analytical specificity	> 99.9995%	> 99.9995%

a. Requires separate, standalone DRAGEN server if secondary analysis with an on-premises server is desired.

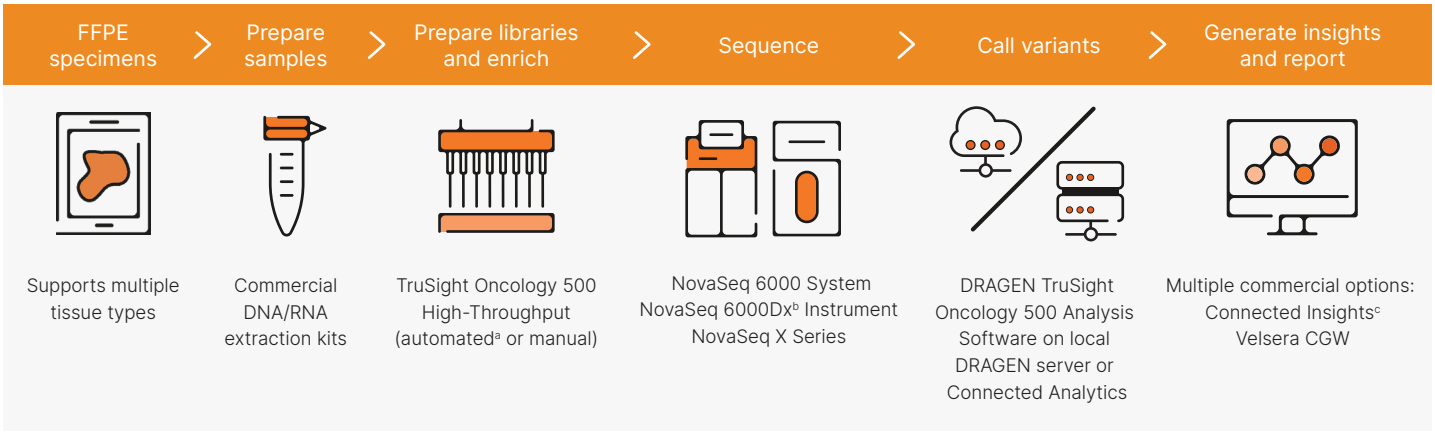

Figure 1: TruSight Oncology 500 High-Throughput workflow—TruSight Oncology 500 High-Throughput integrates easily into current lab workflows, going from nucleic acids to variant calls in four days.

a. TruSight Oncology 500 High-Throughput kits are available in automation-compatible versions.

b. NovaSeq 6000Dx Instrument in research mode.

c. Not available in all countries. Illumina Connected Insights supports user-defined tertiary analysis through API calls to third-party knowledge sources.

Methods

Library preparation

Genomic DNA (gDNA) and RNA samples, previously isolated from FFPE tissue samples (brain, bone, breast, liver, lung, prostate, stomach, thyroid, or uterine tissue) were obtained from on-site Illumina stocks. gDNA and RNA samples were isolated using the AllPrep DNA/RNA FFPE Kit - Nucleic Acid Extraction (QIAGEN, Catalog no. 80234). gDNA input samples were prepared in Tris/Borate/EDTA buffer to a final concentration and volume of 40 ng and 12 µl, respectively. RNA input samples were prepared in RNase/DNase-free water to a final concentration and volume of 80 ng and 8.5 µl, respectively. gDNA and cDNA libraries were prepared from DNA and RNA input samples using the simultaneous DNA/RNA library prep workflow according to the TruSight Oncology 500 High-Throughput Reference Guide (1000000094853-03) using IDT for Illumina UMI DNA/RNA UD Indexes Sets A and B (Illumina, Catalog nos. 20034701 and 20034702, respectively). Pooled gDNA and cDNA libraries were split and pair-matched samples were prepared for sequencing on the NovaSeq X Series and NovaSeq 6000 System.

Sequencing

Matched library samples were sequenced simultaneously on the NovaSeq X Series and the NovaSeq 6000 System. Comparison sequencing runs were performed for the 1.5B versus S1 flow cells, the 10B versus S4 flow cells, and the 25B versus S4 flow cells, using sequencing cycles of 101, 10, 10, 101, at full multiplexing capacity.

Analysis

Sequencing data were demultiplexed using bcl2fastq2 Conversion Software for NovaSeq X v 1.2.05 in BaseSpace™ Sequence Hub and analyzed locally using DRAGEN™ TruSight Oncology 500 Analysis Software 2.5.2. Statistical analyses were performed using JMP statistical analysis software v16 and R 4.3.1. Comparisons between the NovaSeq X Series and the NovaSeq 6000 System data were made across the following measures:

- Baseline assessment
- Limit-of-detection (LOD) and limit-of-blank (LOB) performance
- General concordance for variant calling

Concordance in variant calling between the two systems was evaluated for small variants (SV), copy number variation (CNV), TMB, MSI, RNA fusions, and RNA splice variants.

Results

Instrument performance

Experimental data of sequencing performance demonstrates strong concordance for TruSight Oncology 500 High-Throughput between the NovaSeq X Series and NovaSeq 6000 System (Table 2). As such, changes to the TruSight Oncology 500 High-Throughput assay workflow on the NovaSeq X Series are not necessary. Additionally, sequencing run times on the NovaSeq X Series are shorter than those for the NovaSeq 6000 System, with much higher throughput capacity on the 10B and 25B flow cells (Table 3).

The NovaSeq X System and NovaSeq 6000 System demonstrated similar total passing filter (PF) reads per DNA library between 100–150 million reads (Figure 2).

Table 2: Equivalent performance of TruSight Oncology 500 High-Throughput on the NovaSeq X Series (10B flow cell) and NovaSeq 6000 System (S4 flow cell)

DNA performance metrics	
Metric	Concordance value (R^2)
Contamination score	99%
Median insert size	89%
Median exon coverage	94%
Percent exon bases with 50× fragment coverage	93%
Coverage mean absolute deviation (MAD)	93%
Median bin count CNV targets	97%
RNA performance metrics	
Median CV gene 500×	99%
Total on target reads	94%
Median insert size	99%

Table 3: Comparison of sequencing run times for the NovaSeq X Series and the NovaSeq 6000 System

Sequencing system	Flow cell	Maximum sample throughput[a]	Total run time
NovaSeq X Series	1.5B	32	~18.5 h
	10B	192	~25 h
	25B	480	~ 33 h
NovaSeq 6000 System	S1	32	~19 h
	S2	72	~25 h
	S4	192	~36 h

a. Sample throughout listed is for a single flow cell and both DNA and RNA (ie, the NovaSeq X Series 1.5B flow cell can run 32 DNA samples and 32 RNA samples). Dual flow cell runs are an option available on the NovaSeq X Plus System.

Baseline evaluation

Baselines constructed from normal samples represent an understanding that some sites in the genome are inherently 'noisier' than others. The use of a baseline minimizes noise and improves the signal-to-noise ratio, reducing false positives. A baseline analysis conducted with 77 normal samples demonstrated high concordance in results between the NovaSeq X versus NovaSeq 6000 Systems (Figure 3).

High baseline concordance was demonstrated between both sequencing systems by comparison of false positives (CNVs (R^2 = 0.99) and SVs (R^2 = 0.82)) and MSI classifications (0% false positives), and concordance of TMB per Mb (R^2=0.86). Comparison of small variant frequency (VF) above and below 5% VF demonstrated highly concordant performance for the reported VF limit of detection (LOD) of 0.05. In combination, these analyses demonstrate high baseline concordance between the NovaSeq X Series and NovaSeq 6000 System.

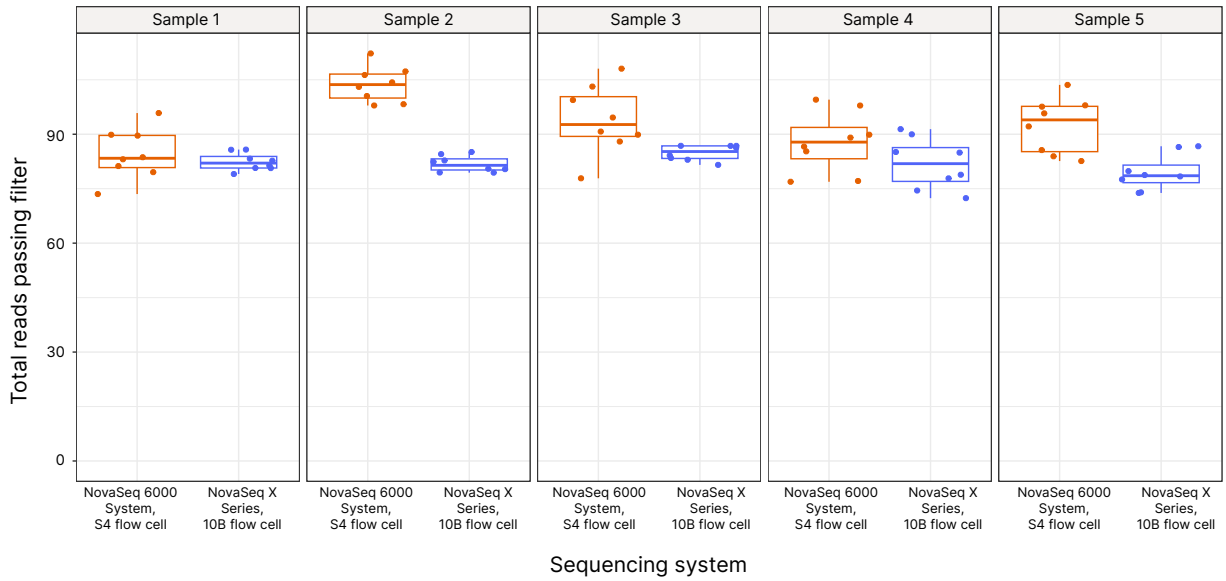

Figure 2: Comparable total reads passing filter (PF) between the NovaSeq X Series and NovaSeq 6000 System at maximum sample throughput—A wide range of FFPE specimens and cell lines was used to prepare 192 DNA and 192 RNA TruSight Oncology 500 High-Throughput libraries, which were then sequenced on the NovaSeq X Series using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell. Replicate libraries run on both sequencers from a subset of five samples is shown.

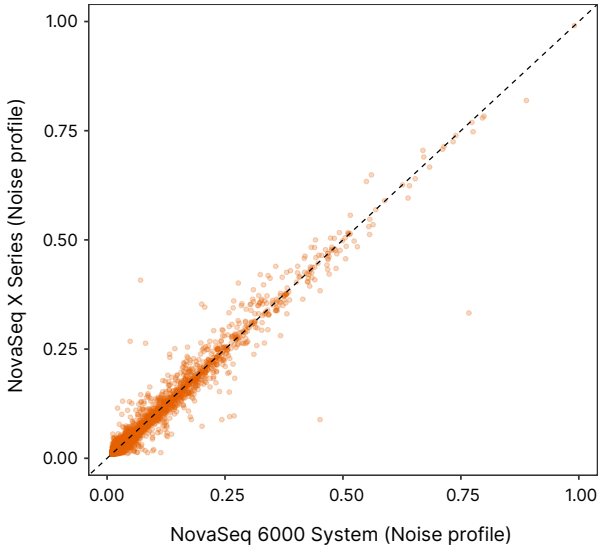

Figure 3: High Concordance between NovaSeq X Series and NovaSeq 6000 System small variant baseline results—Libraries were prepared from 77 normal samples with TruSight Oncology 500 High-Throughput and sequenced on the NovaSeq X Series using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell. Baseline results show a concordance of R^2 = 0.96.

LOD and LOB performance equivalency

Performance equivalency near LOD between the NovaSeq X Series and NovaSeq 6000 System was assessed using 30 samples with known variant calls. Known samples were diluted and mixed with normal cell lines for sequencing analysis, specifically to target < 5% variant allele frequency (VAF). In total, the performance equivalency near LOD between the two systems is strong. LOB was assessed using formalin-fixed paraffin-embedded (FFPE) normal samples (true negatives).

All analytical specificity and sensitivity design inputs pertaining to the TruSight Oncology 500 High-Throughput were met on the NovaSeq X System. The assay demonstrated a small DNA variant specificity of 99.9999% at the variant level, and a specificity of 99.8% for DNA gene amplifications. Assessing cell lines with known variants (from the LOD performance equivalency studies) on the NovaSeq X Series demonstrated a detection rate for small DNA variants of 100% at 5% VAF. LOD sensitivity was 100% for DNA gene amplifications at 2.2-fold change.

Concordance in variant call performance

Performance of TruSight Oncology 500 High-Throughput on the NovaSeq X Series (10B flow cell) and NovaSeq 6000 System (S4 flow cell) demonstrates high concordance (Table 4), with similar analytical specificity (R^2) and sensitivity (positive pairwise agreement (PPA)) across all variant types, including small variants (Figure 4), CNVs (Figure 5), MSI (Figure 6), TMB (Figure 7), RNA fusions (Figure 8), and RNA splice variants (Figure 9). PPA calculations provide a quantitative measure of confidence to the comparisons made between the NovaSeq X Series and the NovaSeq 6000 System, in addition to the R^2 values.

Table 4: High concordance on the NovaSeq X Series and the NovaSeq 6000 System for all variant types

Metric	R^2	PPA
Small variants	99%	91%
CNVs	99%	93%
MSI	98.5%	100%
TMB	98.5%	N/A[a]
RNA fusions	99%	92%
RNA splice variants	97%	77%[b]

a. Positive pairwise agreement score of qualitative variant calls is not applicable as TMB is quantitative only.
b. Discordance for RNA splice variants was due to the NovaSeq X Series obtaining a slightly higher number of supporting reads than the NovaSeq 6000 System in splice variants around the product LOD. Each splice variant had supporting reads from both sequencing systems.

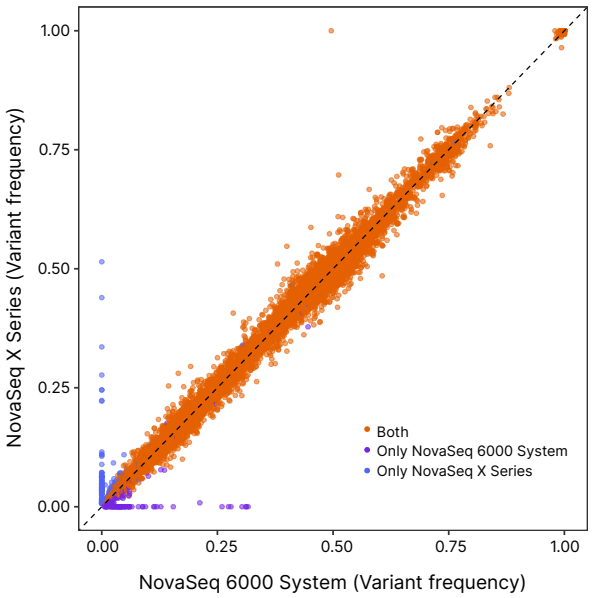

Figure 4: High concordance (R^2 = 99%) for small variant detection—Libraries were prepared from 96 unique DNA samples using TruSight Oncology 500 High- Throughput and sequenced on the NovaSeq X Series using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell. Germline-related variants were filtered with a minimum filter depth at sample level (DP), 100.

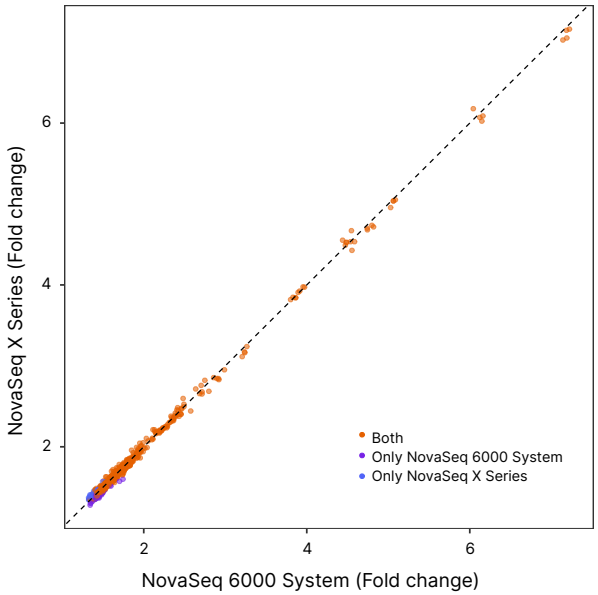

Figure 5: High concordance (R^2 = 99%) for CNV detection—Libraries were prepared from 96 unique DNA samples using TruSight Oncology 500 High-Throughput and sequenced on the NovaSeq X Series using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell.

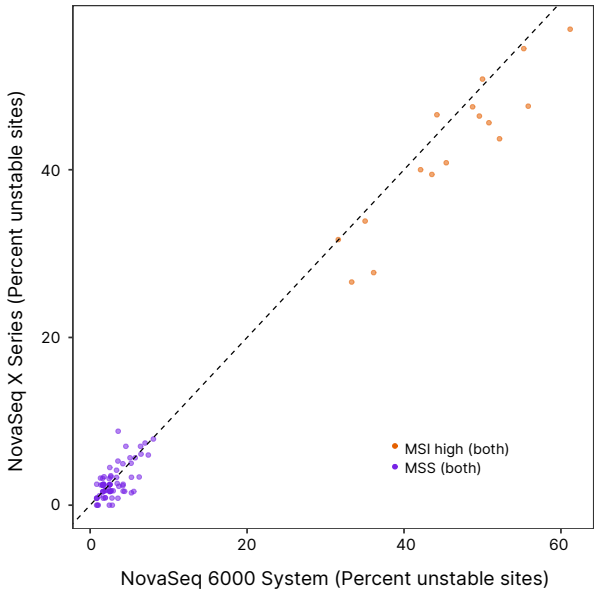

Figure 6: High concordance (R^2 = 98.5%) for MSI detection—Libraries were prepared from 96 unique DNA samples using TruSight Oncology 500 High-Throughput and sequenced on the NovaSeq X Series using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell.

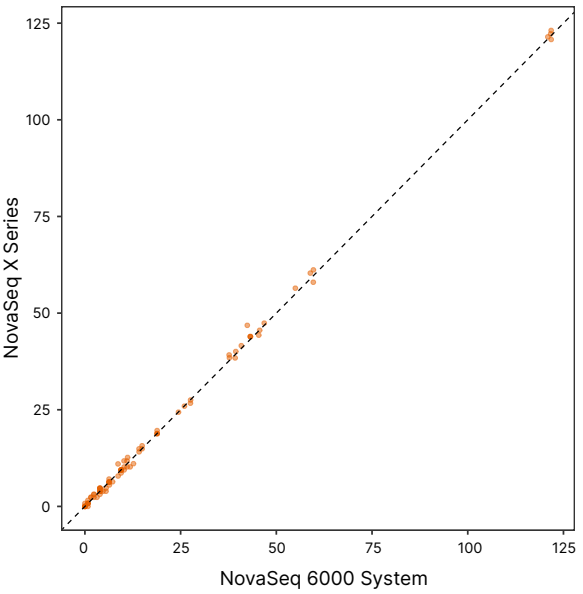

Figure 7: High concordance for (R^2 = 98.5%) TMB detection—Libraries were prepared from 96 unique DNA samples using TruSight Oncology 500 High-Throughput and sequenced on the NovaSeq X System using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell.

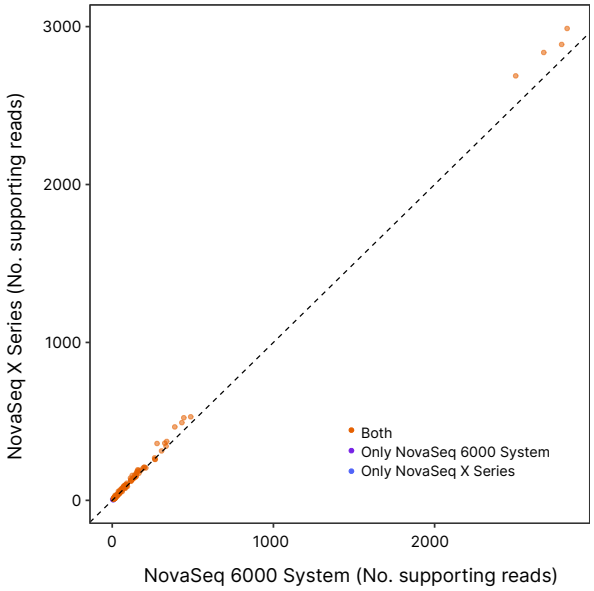

Figure 8: High concordance (R^2 = 99%) for RNA fusion detection—Libraries were prepared from 96 unique RNA samples using TruSight Oncology 500 High-Throughput and sequenced on the NovaSeq X System using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell.

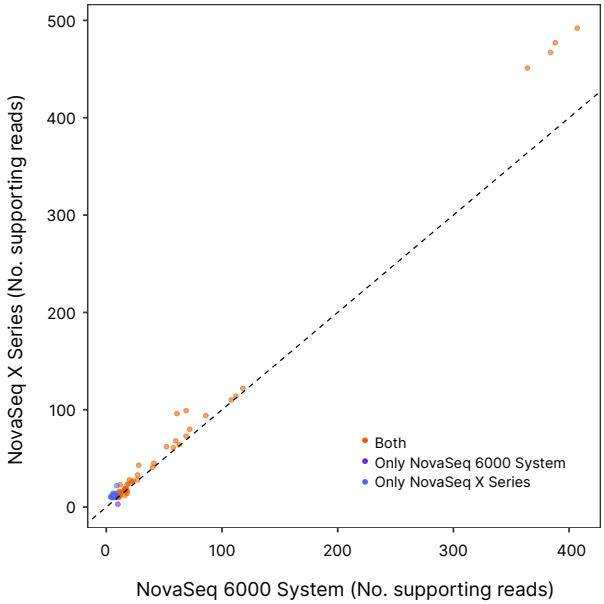

Figure 9: High concordance (R2 = 97%) for RNA splice variant detection—Libraries were prepared from 96 unique RNA samples using TruSight Oncology 500 High-Throughput and sequenced on the NovaSeq X Series using the 10B flow cell and the NovaSeq 6000 System using the S4 flow cell.

Summary

TruSight Oncology 500 High-Throughput has been validated for use on the NovaSeq X Series. Users running TruSight Oncology 500 High-Throughput on the NovaSeq 6000 System can now take advantage of the power and cost savings of the NovaSeq X Series without compromising performance and results. Comparison of TruSight Oncology 500 High-Throughput on the NovaSeq X Series and the NovaSeq 6000 System demonstrates equivalent performance for foundational sequencing performance metrics, as well as concordant results for variant calling.

References

1. Illumina. NovaSeq X and NovaSeq X Plus Sequencing Systems specification sheet. https://www.illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/novaseq-x-series-spec-sheet-m-us-00197/novaseq-x-series-specification-sheet-m-us-00197.pdf. Published 2022. Updated 2024. Accessed August 19, 2024.

Learn more

TruSight Oncology 500 High-Throughput

NovaSeq X Series

DRAGEN secondary analysis

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-02869 v1.0