APPLICATION NOTE

TruSight™ Oncology 500 ctDNA v2 provides superior performance and an improved workflow

TruSight Oncology 500 ctDNA v2 is an improved version of TruSight Oncology 500 ctDNA v1, delivering high-quality, accurate data with:

- Better performance with lower analytical sensitivity—0.2% VAF vs 0.5% VAF for small variants
- Lower input requirements—20 ng vs 30 ng DNA
- Faster library preparation—~8 hrs vs 2.5 days

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Introduction

Comprehensive genomic profiling (CGP) 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] The standard approach for CGP involves use of solid tumor tissue samples, including formalin-fixed paraffin-embedded (FFPE) samples. Sometimes, however, sufficient tissue sample may not be available (this situation can occur up to 25% of the time[3]), the tumor may be inaccessible, or results from tissue biopsy may be delayed. In these cases, performing CGP with circulating tumor DNA (ctDNA) found in the blood, called a liquid biopsy, can provide insights into the genomic landscape of the tumor, often in a faster and less expensive manner than tissue biopsies.[4]

To take advantage of liquid biopsy, it is critical to use a highly sensitive and specific analytical assay capable of detecting somatic mutations present at low frequencies in cfDNA. The original TruSight Oncology 500 ctDNA assay met this challenge, harnessing the power of proven Illumina next-generation sequencing (NGS) technology and achieving the high analytical sensitivity needed to enable CGP. Building on this success, TruSight Oncology 500 ctDNA v2 offers the same comprehensive content with chemistry and workflow improvements that lead to higher analytical sensitivity, faster time to answer, and a more streamlined workflow (Table 1, Table 2, Table 3).[5]

In this technical note, we compare the performance of the TruSight Oncology ctDNA v2 assay with that of the original assay. Data reveal good concordance between results, with improved performance observed in some cases with the v2 assay.

TruSight Oncology 500 ctDNA v2 offers a faster workflow

Both the v1 and v2 TruSight Oncology 500 ctDNA assays follow the same basic steps in the workflow: 1-prepare libraries from cell-free DNA (cfDNA), 2-sequence, 3-analyze data, 4-generate report. The recommended input for TruSight Oncology 500 ctDNA v2 is 20 ng cfDNA, compared to 30 ng for the v1 assay, and requires a single hybridization step, shortening library prep from 2.5 days to 1 day ("Figure 1").

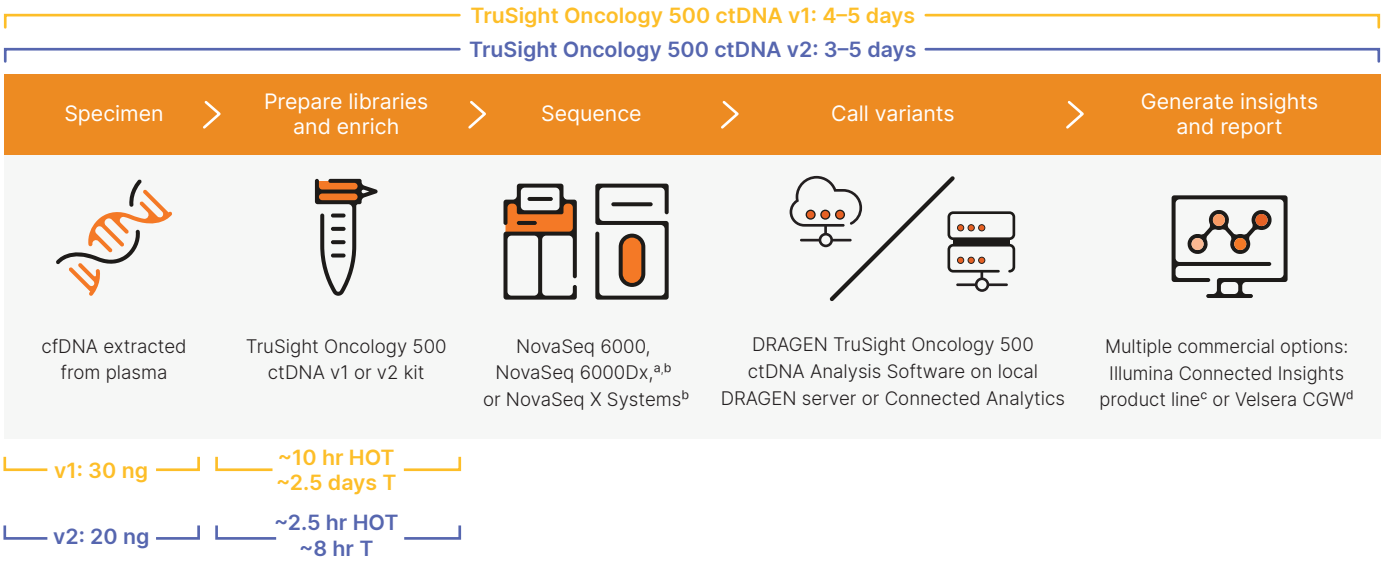


Figure 1: TruSight Oncology ctDNA v2 offers a faster workflow with a lower input requirement compared to the original assay—A single hybridization step during library preparation saves users ~1.5 days, allowing for a shorter workflow duration. HOT, hands-on time. T, total time. a. NovaSeq 6000Dx in RUO mode. b. Available in 2024. c. Available in select countries. Illumina Connected Insights product line supports user-defined tertiary analysis through API calls to third-party knowledge sources. d. Velsera is previously known as Pierian. Other commercial options are available.

Table 1: Shared benefits of TruSight Oncology 500 ctDNA v1 and v2

Benefit	Feature
Comprehensive content	1.94 Mb panel covering 523 genes
Sequencing batch sizes	8 samples (S2 flow cell) 24 or 48 samples (S4 flow cell)
Total sequencing time on the NovaSeq 6000 System	S2 flow cell: ~36 hrs S4 flow cell: ~44 hrs
Total time for secondary analysis[a]	S2 flow cell: ~12 hrs S4 flow cell: ~20 hrs
User-friendly reporting	Multiple commercial options: Illumina Connected Insights product line[b] or third-party options such as Velsera CGW[c]

a. Analysis times shown are for a single flow cell on local DRAGEN server analysis using DRAGEN TruSight Oncology 500 ctDNA v2.1; analysis times may vary significantly using cloud-based Illumina Connected Analytics.
b. Available in select countries. Illumina Connected Insights product line supports user-defined tertiary analysis through API calls to third-party knowledge sources.
c. Velsera is previously known as Pierian. CGW, Clinical Genomic Workspace. Other commercial options are available.

Table 2: Improved user experience with TruSight Oncology 500 ctDNA v2

Benefit of v2	TruSight Oncology 500 ctDNA v2	TruSight Oncology 500 ctDNA v1
Flexible kit sizes	24 or 48 samples	48 samples
Lower input requirement	20 ng recommended (5–10 ng minimum)	30 ng recommended
Faster, single-day library preparation workflow	Single hybridization/capture step • Hands-on time: ~2.5 hrs • Total time: ~8 hrs	Two hybridization/capture steps • Hands-on time: 10 hrs • Total time: ~2.5 days
Faster assay turnaround time	~3.5 days	4–5 days
Streamlined user experience	Plate-based indexes Thermo cycler–based incubation steps	Tube-based indexes Hybex- and thermo cycler–based incubation steps
Automation compatible	Automation-friendly kits and method with flexible batch sizes[a]	No
Increased scalability	192 indexes	16 indexes

a. 48-sample kit is intended for automation.

Demonstrated improvements and data concordance

Methods

Samples

To demonstrate that TruSight Oncology 500 ctDNA v2 yields results equal to, or better than, the original TruSight Oncology 500 ctDNA assay, performance studies were conducted using control samples from LGC Clinical Diagnostics or real-world clinical samples* (Table 4). Control samples were provided as extracted nucleic acids and did not require further preparation for use in the TruSight Oncology 500 ctDNA assays. Nucleic acid from the clinical samples tested by Illumina was extracted using the QIAamp Circulating Nucleic Acid Kit (QIAGEN, Catalog no. 55114).† Nucleic acid from the clinical samples tested by early access sites was extracted using either the MagMAX Cell-Free DNA Isolation Kit (Thermo Fisher Scientific, Catalog no. A29319) or QIAsymphony DSP Circulating DNA Kit (QIAGEN, Catalog no. 937556).

* Human plasma provided for research use only.

† Data presented reflects use of the QIAamp Circulating Nucleic Acid Kit for DNA extraction. Equivalent sequencing performance was achieved using QIAamp kits (QIAGEN), Quick-cfDNA (Zymo Research), or Apostle MiniMax High Efficiency Cell-Free DNA Isolation Kit (Beckman Coulter) (data not shown).

Table 3: Improved performance with TruSight Oncology 500 ctDNA v2

Benefit of v2	TruSight Oncology 500 ctDNA v2	TruSight Oncology 500 ctDNA v1
Increased analytical sensitivity (SNVs)	95% sensitivity at 0.4% VAF (20 ng input) 90% sensitivity at 0.2% VAF (20 ng input)	95% sensitivity at 0.5% VAF (30 ng input)
Increased analytical specificity	> 99.995% for SNVs > 95% for all other variants	≥ 95%
Advanced secondary analysis	DRAGEN TruSight Oncology 500 ctDNA v2 Analysis Software v2.1+, including improved CH filtering	DRAGEN TruSight Oncology 500 ctDNA pipelines

Library preparation

Libraries were prepared manually following the protocols specified in the user guides for each TruSight Oncology 500 ctDNA assay (Table 5).

Sequencing

For both assays, sequencing was performed on the NovaSeq™ 6000 Sequencing System (Table 6).

Analysis

Data analysis for both assays was performed using the DRAGEN™ TruSight Oncology 500 ctDNA v2.1 pipeline on a local DRAGEN server. Additional analysis of variant calls and QC metrics was performed using JMP statistical software, R, and Excel.

Table 4: Samples used for comparison studies

Control samples purchased from LGC Clinical Diagnostics	
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
SeraSeq ctDNA Mutation Mix AF 1%	0710-0140
SeraSeq ctDNA Mutation Mix AF 0.5%	0710-0141
SeraSeq ctDNA Mutation Mix AF 0.25%	0710-0142
SeraSeq ctDNA Mutation Mix AF 0.125%	0710-0143
Real-world clinical samples: De-identified cancer patient specimens	
Samples purchased from Biospecimen vendors or samples consented for research use received as part of regular practice from early access sites included the following cancer types: breast, cervical, colorectal, head and neck, lung (including non-small cell lung cancer), ovarian, and prostate.	

Table 5: Library preparation parameters

Kit	Catalog no.	Input amount per sample
TruSight Oncology 500 ctDNA	20039252	30 ng
TruSight Oncology 500 ctDNA v2	20105899	20 ng

Table 6: Sequencing run parameters on the NovaSeq 6000 Sequencing System

Parameter	Value
Read length	2 × 151 bp
Index length	v1: 8 bp, dual indexed v2: 10 bp, dual indexed
No. of cycles	300
No. of reads	~400M clusters per sample

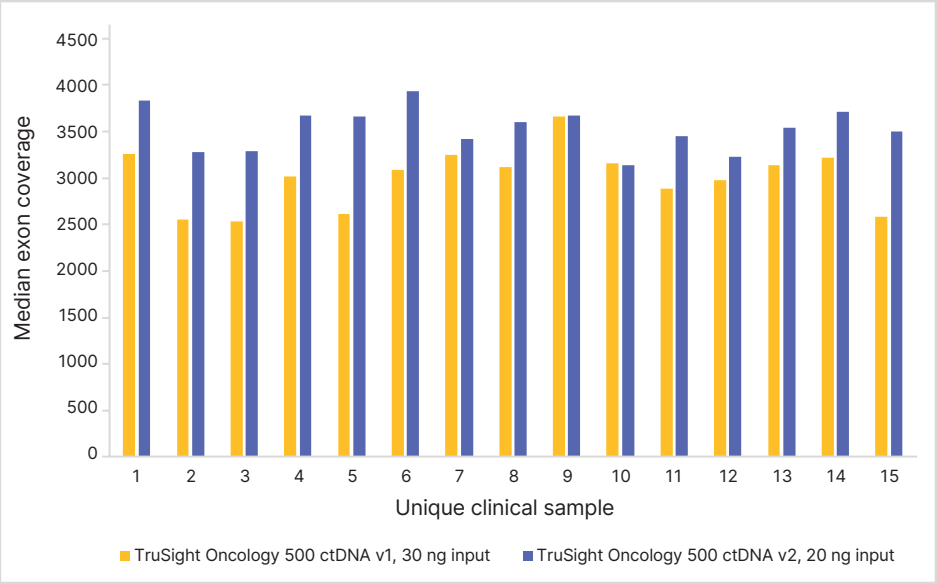
Results

Higher median exon coverage with less sample

Median exon coverage (MEC) measures the number of reads spanning an exon region. The higher the MEC, the higher the confidence in the sequencing data produced.

Clinical samples analyzed using the TruSight Oncology 500 ctDNA v1 or v2 assays showed a higher MEC for clinical samples prepared using the v2 assay for both internal (Illumina) and external (early access) sites (Figure 2), which is due to improvements in library conversion efficiency. Note that this was achieved using 33% less input cfDNA in the v2 assay (20 ng in v2 vs 30 ng in v1).

A. Data generated by Illumina using clinical samples



B. Data generated by external early access sites using clinical samples

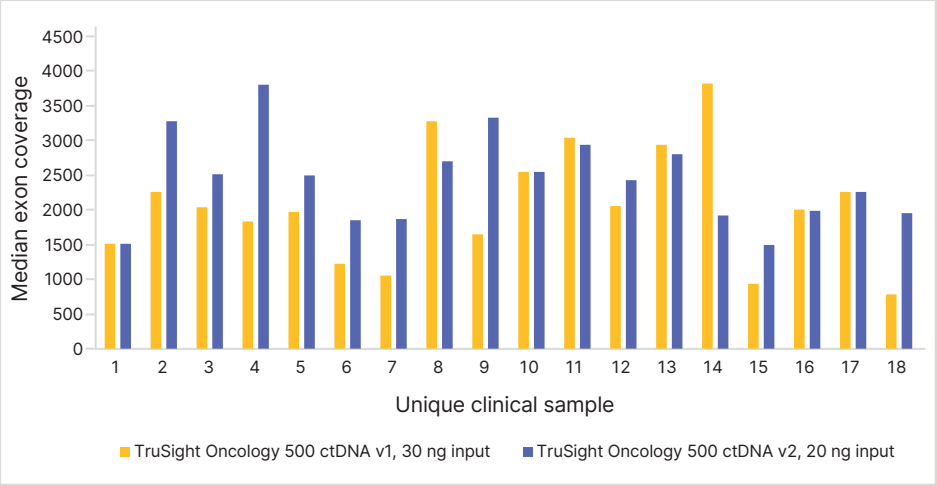
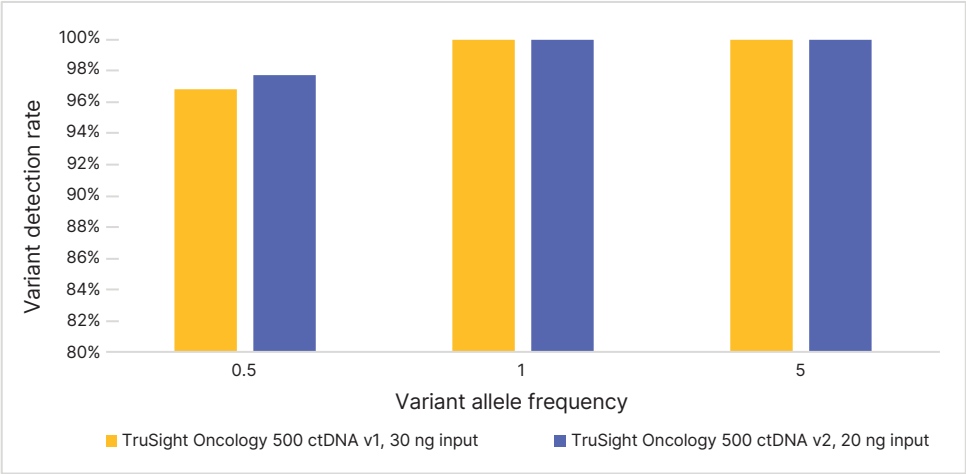


Figure 2: Higher MEC observed when analyzing clinical samples with TruSight Oncology ctDNA v2 vs v1, even with less cfDNA input—Various clinical samples with known cancer types were analyzed by Illumina and external labs using TruSight Oncology 500 ctDNA using 30 ng input ctDNA or TruSight Oncology 500 ctDNA v2 using 20 ng of ctDNA. Each site used different clinical samples.

Better small DNA variant detection

TruSight Oncology 500 ctDNA assays are designed to provide accurate and sensitive detection of low-level biomarkers. For small DNA variants, the v1 assay has an analytical sensitivity of ≥ 95% at 0.5% variant allele frequency (VAF) (based on 30 ng input cfDNA) and the v2 assay has an analytical sensitivity of ≥ 90% at 0.2% VAF (based on 20 ng input cfDNA). Comparison studies using SeraSeq ctDNA Complete Mutation Mix control samples performed across multiple external sites showed high concordance of small DNA variant detection at > 0.5% VAF (Figure 3A). Studies performed by one external site using replicates of the SeraSeq Mutation Mix v2 control to target lower variant allele frequencies further show higher sensitivity at VAFs below 0.5% using less input (Figure 3B).

A. Data aggregate across multiple labs with SeraSeq control samples



B. Data aggregate across multiple users with SeraSeq control samples

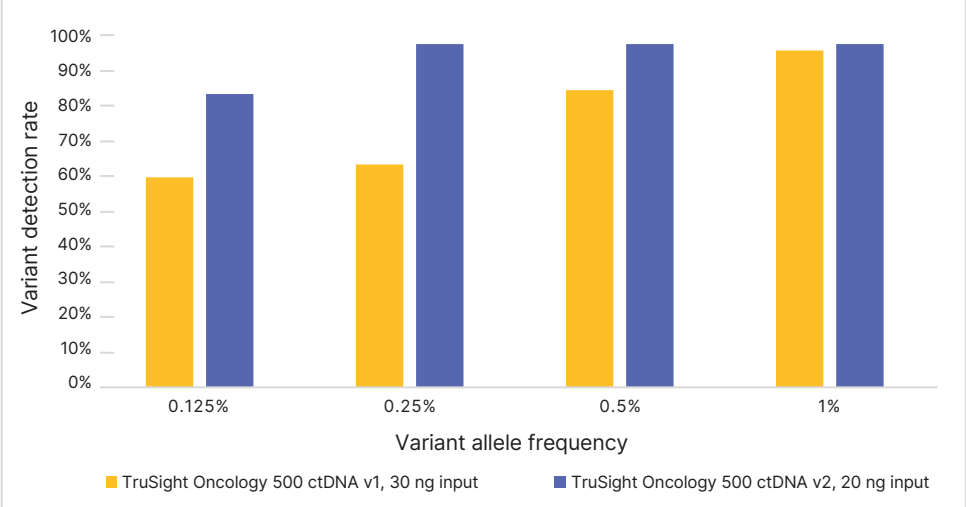


Figure 3: Improved detection for small variants at VAF < 0.5%—(A) SeraSeq ctDNA Complete Mutation Mix AF 0.5% control samples were analyzed by multiple external labs using TruSight Oncology 500 ctDNA with 30 ng input ctDNA (four labs) and TruSight Oncology 500 ctDNA v2 with 20 ng of ctDNA (seven labs). (B) SeraSeq Mutation Mix v2 control samples tested in singleton by three operators at one external lab using TruSight Oncology 500 ctDNA with 30 ng input ctDNA and TruSight Oncology 500 ctDNA v2 with 20 ng of ctDNA. Sensitivity for small DNA variants, particularly below 0.5% VAF is improved in the v2 assay relative to the original assay.

Similarly, studies performed with clinical samples showed good small variant detection concordance at > 0.5%, with 86.8% of all small DNA variants called in the v1 assay also detected in the v2 assay (Table 7)‡. Further evaluations of samples tested internally show a higher sensitivity using the v2 assay for variants with VAF below 0.5% VAF (Figure 4). Studies were also performed by external labs using clinical samples with TruSight Oncology 500 ctDNA v1 or TruSight Oncology 500 ctDNA v2. Results indicate that for some variants, particularly variants at lower input (ie, 10 ng, 5 ng), detection was achievable with the v2 assay, but variant calls were missed in the v1 assay (Table 8).

High bTMB concordance

Tumor mutational burden (TMB or bTMB when from a blood-based sample), or the number of nonsynonymous mutations within the coding region of a tumor genome, is an emerging biomarker that correlates with response to immunotherapeutic agents such as checkpoint inhibitors.[6-8] bTMB analysis in clinical samples including lung, colorectal and ovarian cancer, with TruSight Oncology 500 ctDNA v1 and v2 shows high concordance (Figure 5).

‡ See Table 7 footnote..

Table 7: Good small variant concordance in clinical samples at > 0.5% VAF

Variant type	Percent of variants called in v1 also detected by v2
All small variants	86.8%
SNVs	88.8%
Insertions/deletions	71.4%

TruSight Oncology 500 ctDNA v2 analysis is performed using the DRAGEN TruSight Oncology 500 ctDNA v2 analysis software. This software offers improved germline proxy filtering and CH filtering compared to the original DRAGEN pipeline used for analysis of the v1 assay. Due to these enhancements, some of the variants called in the v2 assay are more accurately classified and will be noted as germline or CH variants and not fall within the categories of small variants, SNVs, or indels noted in the table.

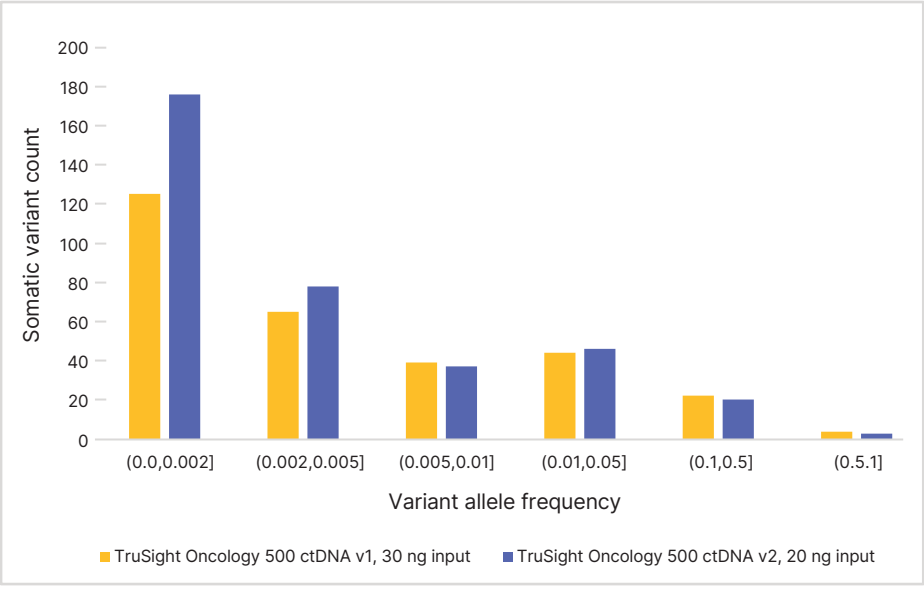


Figure 4: Improved detection of small DNA variants in clinical samples at VAF < 0.5% using TruSight Oncology 500 ctDNA v2.

Table 8: TruSight Oncology 500 ctDNA v2 show higher sensitivity for variant calls < 2% VAF in real-world clinical samples

				TruSight Oncology 500 ctDNA v2		TruSight Oncology 500 ctDNA v1	
Sample no.	Input amount[a]	Gene	Variant	VAF	Depth	VAF	Depth
1	20 ng/30 ng	*ALK*	p.G1202R	0.81%	2980	0.69%	3352
2	20 ng/30 ng	*SMARCA4*	p.Q194fs*93	1.37%	1754	1.09%	827
2	20 ng/30 ng	*KEAP1*	p.K97*	1.71%	2287	1.62%	2470
2	20 ng/30 ng	*STK11*	p.L245F	1.76%	1190	2.20%	318
3	20 ng/30 ng	*TP53*	p.C238fs*9	0.60%	2822	1.18%	2802
3	20 ng/30 ng	*PIK3CA*	p.E545G	0.64%	2795	0.48%	2897
3	20 ng/30 ng	*KRAS*	p.G12C	1.93%	2179	1.91%	2519
4	20 ng/30 ng	*TP53*	p.H193Y	0.11%	1838	0.11%	3736
5	20 ng/30 ng	*PIK3CA*	p.H1047R	0.23%	1310	0.25%	839
5	20 ng/30 ng	*TP53*	p.R248W	0.26%	1550	0.18%	1087
6	20 ng/30 ng	*CHEK2*	p.E457*	0.39%	1548	0.49%	1635
7	20 ng/30 ng	*EGFR*	p.E746_A750del	0.65%	2271	0.72%	1250
7	10 ng	*EGFR*	p.E746_A750del	0.08%	1217	Not called	–
7	5 ng	*EGFR*	p.E746_A750del	1.08%	554	Not called	–
8	20 ng/30 ng	*BRCA2*	p.K2170*	0.87%	1486	2.89%	173
8	20 ng/30 ng	*NBN*	p.R632fs*6	1.64%	2262	0.68%	293
8	20 ng/30 ng	*TP53*	p.R248Q	1.73%	2250	0.29%	342
8	10 ng	*BRCA2*	p.Q2164_S2172del	0.72%	1104	Not called	–
8	10 ng	*TP53*	p.R248Q	0.92%	650	Not called	–
8	10 ng	*BRCA2*	p.K2170*	0.98%	815	Not called	–
8	10 ng	*NBN*	p.R632fs*6	1.44%	1248	Not called	–
8	10 ng	*TP53*	p.R213*	1.54%	1103	Not called	–
8	5 ng	*BRCA2*	p.Q2164_S2172del	0.52%	578	Not called	–
8	5 ng	*BRCA2*	p.K2170*	0.91%	438	Not called	–
8	5 ng	*NBN*	p.R632fs*6	1.81%	662	Not called	–

a. Input amount of 20 ng cfDNA was used for TruSight Oncology 500 ctDNA v2 assays, while 30 ng cfDNA was used for TruSight Oncology 500 ctDNA v1. Input amounts listed at 5 ng and 10 ng indicate the input quantity used in both assays.

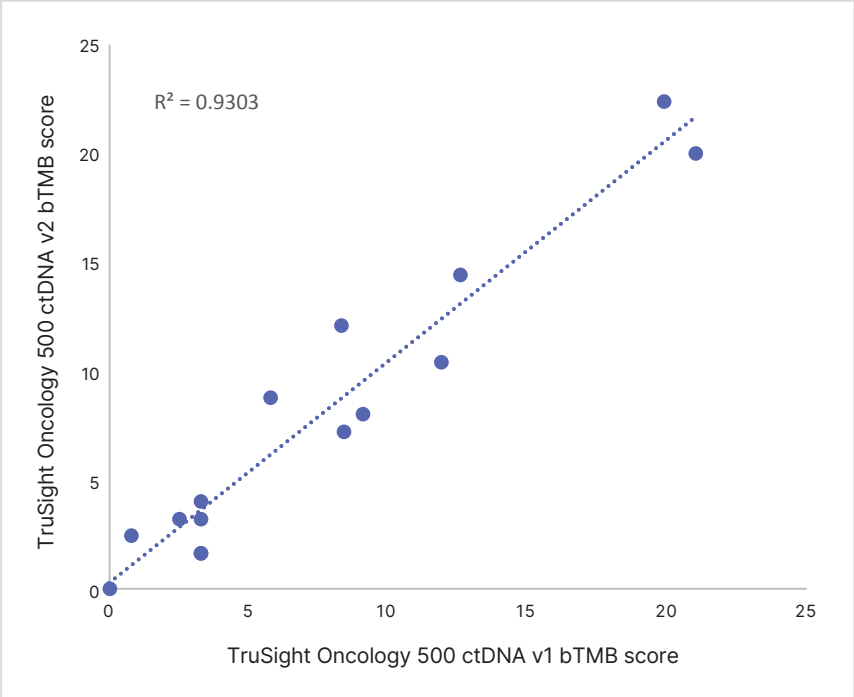


Figure 5: High bTMB concordance in clinical samples tested in house at Illumina—Various clinical samples with known cancer types were analyzed in an Illumina laboratory using TruSight Oncology 500 ctDNA with 30 ng input ctDNA and TruSight Oncology 500 ctDNA v2 with 20 ng of ctDNA for the ability to detect bTMB.

Conclusion

TruSight Oncology 500 ctDNA v2 offers the same comprehensive content as the original v1 assay with significant advantages—faster workflow, lower input requirements, and improved detection of small variants at lower VAF levels (0.2% vs 0.5%). High concordance between the assays is observed for data generated using clinical or control samples.

Learn more

TruSight Oncology 500 ctDNA v2

References

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