DATA SHEET

TruSight™ Oncology 500 ctDNA v2

Enabling sensitive, fast comprehensive genomic profiling from liquid biopsy samples

- Detect biomarkers present as low as 0.2% VAF from 20 ng ctDNA
- Obtain comprehensive results in < 4 days with manual or automated* options
- Analyze > 500 genes and immuno-oncology (IO) genomic signatures (MSI, TMB) in one assay

* Automation-friendly kits and methods available in 2024.

illumina®

CGP and liquid biopsy

Understanding the genomic basis of cancer can help pinpoint alterations that fuel the disease and enable advancements in precision medicine. One method for approaching these oncology studies is comprehensive genomic profiling (CGP). 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] In addition, CGP tests can identify more clinically relevant variants than conventional testing approaches, such as single-gene tests and hotspot NGS panels.[3-6] This ability to detect more variants grows in importance as an increasing number of biomarkers are being discovered, including IO genomic signatures such as tumor mutational burden (TMB) that require large NGS panels (> 1 Mb) for accurate identification.[7,8]

The standard approach for CGP involves use of solid tumor tissue samples, including formalin-fixed paraffin-embedded (FFPE) samples. In some cases, however, sufficient tissue sample may be unavailable (this can occur up to 25% of the time[9]), the tumor may be inaccessible, or results from tissue biopsy may be too delayed. In these cases, performing CGP with circulating tumor DNA (ctDNA) from a liquid biopsy can provide insights into the genomic landscape of the tumor. Liquid biopsy:

- provides a noninvasive approach to obtaining cell-free DNA (cfDNA), which includes ctDNA, from blood plasma for tumor profiling (Figure 1)
- enables access to circulating tumor DNA (ctDNA) that can represent clones from multiple tumors or even the same tumor,[10] overcoming inherent sampling bias present with tumor biopsy and expanding the ability to identify more alterations[10-13]
- is increasingly being included in professional guidelines (> 12 diseases) as a method for obtaining sample for molecular profiling[14-16]

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[17] met this challenge, harnessing the power of proven Illumina NGS technology and achieving the high analytical sensitivity needed to enable CGP. Building on this success, TruSight Oncology 500 ctDNA v2 offers chemistry and workflow improvements that lead to higher sensitivity and a faster time to answer (Table 1, Table 2, Table 3).

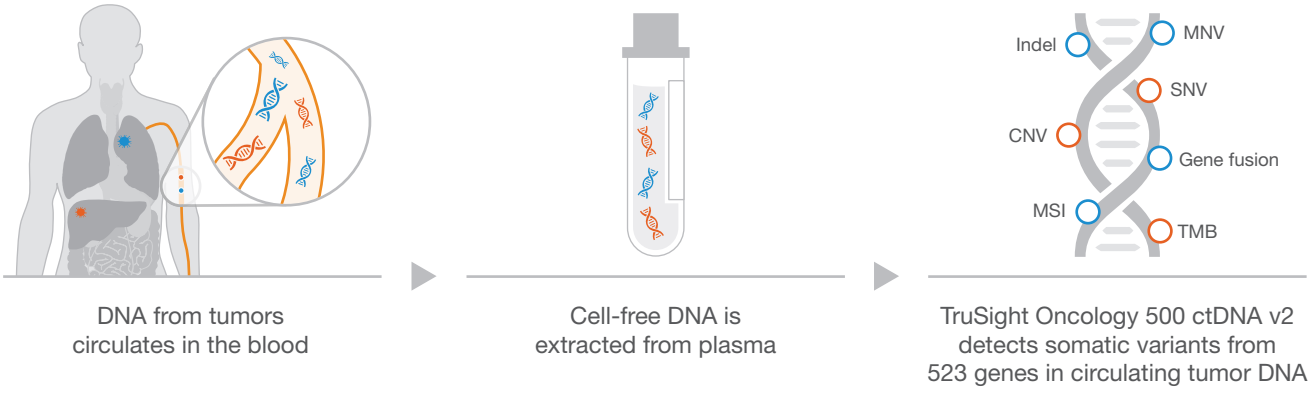

Figure 1: Liquid biopsy enables a noninvasive approach for CGP—ctDNA, found in cell-free DNA in plasma, can be obtained through a simple blood draw analyzed using TruSight Oncology 500 ctDNA v2 to detect the presence of cancer-relevant biomarkers in key guidelines.

The value of ctDNA for CGP

Tumors shed DNA into the peripheral blood upon apoptosis or necrosis, where it circulates as a fraction of the total cell-free DNA (cfDNA).[18] ctDNA has been detected through all stages of cancer progression and across multiple solid tumor types,[18] including lung, breast, colorectal, and ovarian cancers. For certain diseases, such as NSCLC, adding CGP analysis from liquid biopsy to tissue analysis can increase the identification of clinically relevant mutations by 15–48%.[11,12,19] In addition, non-small cell lung cancer studies have revealed that cfDNA analyses are highly concordant with tissue-based analyses.[12]

Using ctDNA for CGP has several advantages:

- Access sample easily through a minimally invasive blood draw procedure[20]
- Obtain temporal and spatial information about intra- and inter-tumor heterogeneity[20]
- Repeat analysis to assess clonal selection

Table 1: Advances with TruSight Oncology ctDNA v2

Benefit	TruSight Oncology 500 ctDNA v2	TruSight Oncology 500 ctDNA (original)
Improved assay sensitivity	Separate end-repair and A-tailing steps	Combined end-repair and A-tailing
More streamlined workflow and improved user experience	Plate-based indexes/UMI	Tube-based indexes/UMI
Faster, single day workflow	Single hybridization/ capture step	Two hybridization and capture steps
More scalability	192 indexes	16 indexes

UMI, Unique molecular identifier.

Learn more about UMIs.

Table 2: TruSight Oncology ctDNA v2 at a glance

Parameter	Specification
System	NovaSeq 6000 System NovaSeq 6000Dx Instrument (RUO mode)[a] NovaSeq X Sequencing System[a]
Sample throughput	8, 24, or 48
Library preparation kit sizes	24-sample (manual) 48-sample (automated)
Sequencing batch sizes	8, 24, or 48 samples
Automation capability	Illumina Qualified methods available for the Hamilton Microlab STAR[a]
Panel size	1.94 Mb DNA
Panel content	523 genes for small variants 59 genes for CNVs 23 genes for gene rearrangements MSI (> 2300 loci) TMB (> 1 Mb)
Sample type	cfDNA derived from blood plasma
DNA input requirement	20 ng cfDNA (5–30 ng possible)[b]
Total assay time	< 4 days from library prep to variant report
Hands-on time	8–24 samples (manual): ~2.5 h 48 samples (automated): TBD[c]
Library preparation time	8–24 samples (manual): < 8.5 h 48 samples (automated): TBD[c]
Sequence run time	8 samples: 36 h (S2 flow cell) 24 samples: 44 h (1 × S4 flow cell) 48 samples: 44 h (2 × S4 flow cell)
Sequence run read length	2 × 151 bp
Sequencing coverage	35,000×
Variant analysis time	8 samples: 9-12 h 24 samples: 20-24 h 48 samples: TBD[c]

a. Available in 2024.
b. Recommend quantification with Agilent TapeStation or Fragment Analyzer systems.
c. To be determined. Available in 2024.

Table 3: TruSight Oncology ctDNA v2 performance

Parameter	Specification
Limit of detection (LOD)	0.2% VAF for SNVs 0.5% VAF for MNVs and indels 0.5% VAF for gene rearrangements ≥ 1.3-fold change for gene amplifications ≤ 0.6-fold change for gene deletions ≥ 0.3% tumor fraction for MSI
Analytical sensitivity (at LOD)	≥ 90% (at LOD of 0.2% VAF for SNVs) ≥ 95% (at LOD of 0.2% VAF for SNV hotspots) ≥ 95% (at LOD of 0.5% VAF for all other variant types)
Analytical specificity	≥ 99.999%

Comprehensive content

Content for TruSight Oncology 500 ctDNA v2 was designed with recognized authorities in the oncology community and includes current and emerging biomarkers with comprehensive coverage of genes involved in key guidelines and clinical trials for multiple tumor types. The panel probe design captures both known and novel gene rearrangements and includes 523 genes for detecting variants likely to play a role in tumorigenesis now and into the future. Biomarkers comprise single-nucleotide variants (SNVs), multi-nucleotide variants (MNVs), insertions/deletions (indels), copy-number variants (CNVs), gene rearrangements, and complex IO genomic signatures, such as blood-based microsatellite instability (bMSI) and blood-based TMB (bTMB) (Table 4).

For a complete list of genes, visit the TruSight Oncology 500 ctDNA v2 product page.

Table 4: Examples of variant types detected by TruSight Oncology 500 ctDNA v2

Variant type	Example
SNVs and indels	*EGFR*, *POLE*, *TMPRSS2*, *BRAF*
Gene rearrangements	*ALK*, *ROS1*, *NTRK1*, *NTRK2*, *RET*
CNVs	*HER2*
MSI	MSI-Score
TMB	TMB-Score

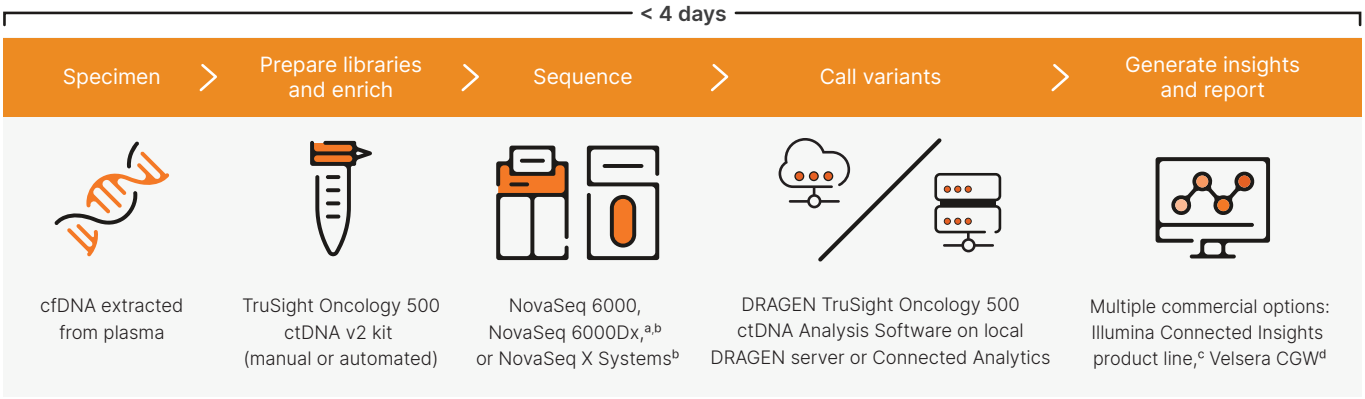

Figure 2: TruSight Oncology 500 ctDNA v2 workflow—TruSight Oncology 500 ctDNA v2 integrates into current lab workflows, going from cfDNA to a variant report in less than four days. DRAGEN TruSight Oncology 500 ctDNA Analysis Software runs locally on a DRAGEN Server or in the cloud version via Illumina Connected Analytics. a. NovaSeq 6000Dx 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.

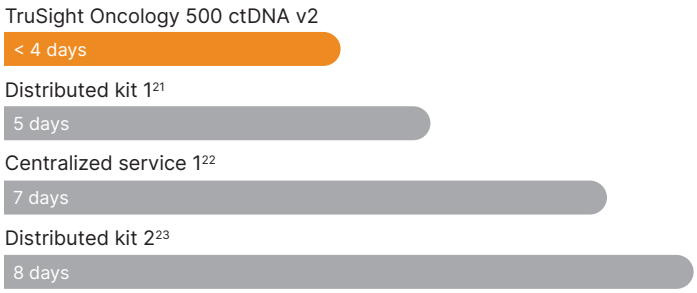

Figure 3: Faster time to report with TruSight Oncology 500 ctDNA v2—A comparison of the time required to go from sample to report for liquid biopsy CGP assays that include the IO biomarkers of bMSI and bTMB.

Fast, integrated workflow

TruSight Oncology 500 ctDNA v2 is part of an integrated CGP workflow that spans from sample input to final report (Figure 2). Using automated library preparation kits and methods, variant calling tools, and interpretation and reporting software enables a smooth workflow that can be completed in less than four days, less than half the time of other CGP liquid biopsy assays (Figure 3).

Optimized library preparation

Using proven Illumina sequencing by synthesis (SBS) chemistry, TruSight Oncology 500 ctDNA v2 enables comprehensive genomic profiling from just 20 ng cfDNA, making it an ideal alternative for use when tissue is not readily available or as a complement to tissue analysis. ctDNA represents a small fraction of cfDNA (often < 5% of total cfDNA), requiring powerful methods to separate signal from noise. To enable ultra-low frequency variant identification, library preparation takes advantage of target enrichment with biotinylated probes and streptavidin-coated magnetic beads to enrich for selected targets from DNA-based libraries and unique molecular identifiers (UMIs)[24] to reduce error rates (Figure 4). Advances in product chemistry have decreased the number of hybridizations from two down to one in TruSight Oncology 500 ctDNA v2, allowing for a one-day turnaround for library preparation and a shorter time to results. It has also improved analytical sensitivity down to 0.2% VAF for SNVs. This targeted hybridization–capture approach reduces sample dropouts in the presence of both natural allelic variations and sequence artifacts.

Automation-enabled workflow

TruSight Oncology 500 ctDNA v2 offers manual and automated* options to support scalable library prep. Illumina has partnered with Hamilton, a leading liquid-handling manufacturer, to produce a fully automated workflow for TruSight Oncology 500 ctDNA v2 assays on the Hamilton Star. Illumina will offer automation-friendly library preparation kits that reduce reagent waste, ensuring sufficient reagent is available for preparing 48 libraries and accommodating for the dead volume required for the robot. Automated workflows achieve the same high-quality results produced by manual protocols, while reducing hands-on time, enabling labs to save on labor costs and improve efficiency.[26]

Powerful sequencing

TruSight Oncology 500 ctDNA v2 libraries are sequenced on the NovaSeq 6000 Sequencing System, NovaSeq 6000Dx Instrument (RUO mode), and NovaSeq X Sequencing System† at high depth (400M reads per sample at ~35,000×) to enhance sensitivity. The result is the ability to detect mutations at 0.2% variant allele frequency (VAF) for SNVs, with ≥ 90% analytical sensitivity and ≥ 95% analytical specificity (Table 5).

* Illumina Qualified automation method available in 2024.

† Sequencing on the NovaSeq 6000 Dx and NovaSeq X Systems available in 2024.

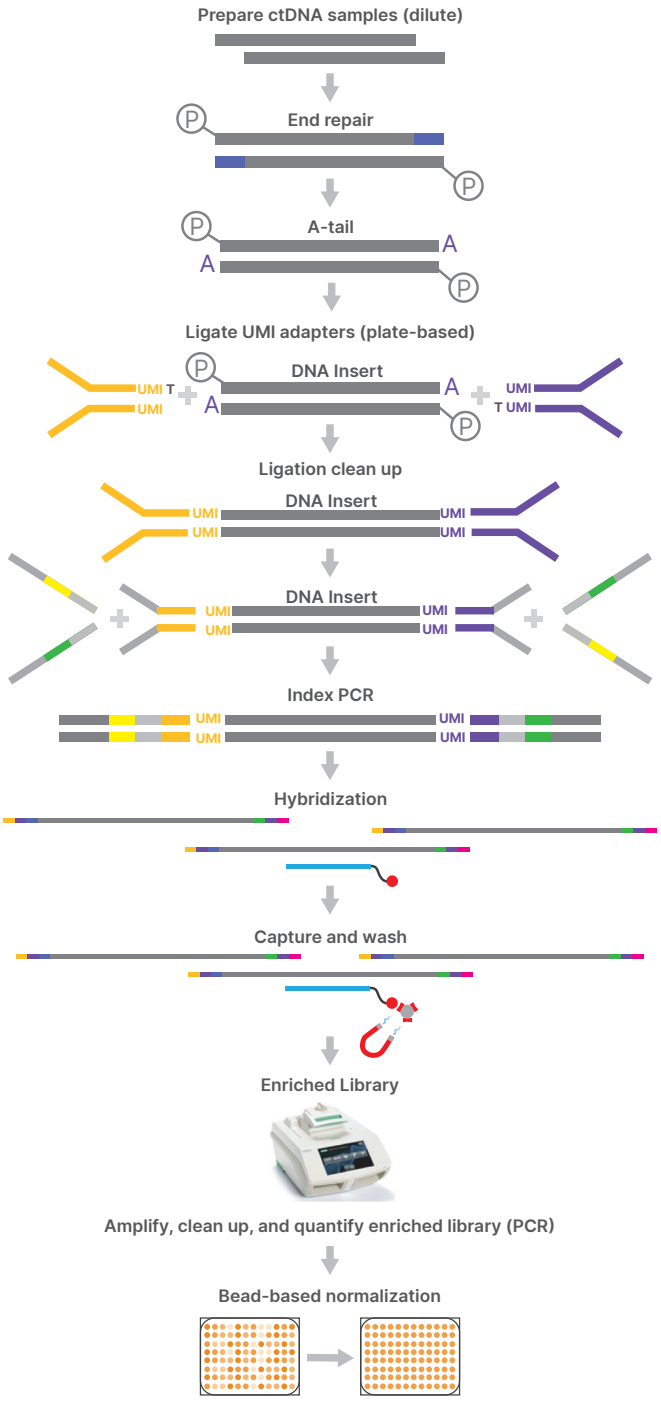

Figure 4: UMI-based hybrid–capture library preparation—Targeted enrichment uses probes large enough to impart high binding specificity, but still allow hybridization to targets containing mutations. UMI reagents reduce error rates, increasing analytical specificity and yielding higher confidence variant calls.[25]

Table 5: Accurate detection of low-level biomarkers

Variant type	Analytical sensitivity[a]	Analytical specificity[b]
Small nucelotide variants (≥ 0.2% VAF)	≥ 90%	≥ 99.9994%
Multi-nucelotide variants (≥ 0.5% VAF)	≥ 90%	≥ 95%
Insertions/deletions (≥ 0.5% VAF)	≥ 90%	≥ 95%
Gene amplifications (≥ 1.3-fold change)	≥ 95%	≥ 95%
Gene deletions (≤ 0.6-fold change)	≥ 95%	≥ 95%
Gene rearrangements (≥0.5%)	≥ 95%	≥ 95%
MSI high detection (≥ at 0.3% tumor fraction)	≥ 95%	≥ 95%

a. Analytical sensitivity is defined as percent detection at the stated variant level.
b. Analytical specificity is defined as the ability to detect a known negative.

Accurate, accelerated analysis

Comprehensive, efficient variant calling

The DRAGEN™ TruSight Oncology 500 ctDNA Analysis pipeline uses accelerated, fully integrated bioinformatics algorithms to perform sequence alignment, error correction by collapsing the sequence, then variant calling based on the raw data. Duplicated reads and sequencing errors are removed without losing signal for low-frequency variants while yielding high-sensitivity variant calling results.

Unlike qualitative results from PCR-based assays, DRAGEN TruSight Oncology 500 ctDNA Analysis pipeline provides a quantitative bMSI score derived from > 2300 homopolymer MSI marker sites. For bTMB analysis, the DRAGEN pipeline optimizes sensitivity by measuring both nonsynonymous and synonymous SNVs and indels. After variant calling and error correction, the accuracy of bTMB measurement is further enhanced by filtering germline variants, low-confidence variants, and variants associated with clonal hematopoiesis of indeterminate potential.

DRAGEN TruSight Oncology 500 ctDNA Analysis pipeline runs locally on an Illumina DRAGEN Server v4 or in the cloud via Illumina Connected Analytics (ICA). ICA offers a secure, cloud-based genomics platform to scale secondary analysis without the need to acquire and maintain local infrastructure.[27] Enhanced DRAGEN hardware and software that reduce data analysis time by ~85% (Table 6).

Table 6: Reduced time for data analysis for 24 samples using an S4 flow cell

Data analysis step	Solution A[a]	DRAGEN TruSight Oncology 500 ctDNA Analysis pipeline
BCL conversion	6 hr	1 hr
Alignment + collapsing + realignment	170 hr	11 hr
Gene rearrangement calling	10 hr	2 hr
Variant calling	24 hr	8 hr
Total time	~9 days	~20 hr (**~85% reduction**)

a. Single node (128G memory, 24 cores CPU), nonparallelized pipeline.

Streamlined data interpretation

After variant class and biomarker type identification via secondary analysis, the next step is to interpret the data to extract biologically relevant meaning. Illumina Connected Insights,‡ Velsera Clinical Genomics Workspace,§ and third-party apps can be used.

Variant calling files produced locally or via the cloud with Illumina Connected Analytics can be automatically ingested into Illumina Connected Insights. When combined with sequencing system integration and the autolaunch capabilities of Connected Analytics, the analysis workflow can be fully automated with Connected Insights, removing the need for manual data transfers, resulting in a final customizable report.

Reliable, reproducible results

TruSight Oncology 500 ctDNA v2 provides sensitive detection of genomic variants and biomarkers in a cfDNA sample, even when present at low levels. To demonstrate the high-quality results achieved with TruSight Oncology 500 ctDNA v2, Illumina performed various studies evaluating the ability to call small DNA variants, CNVs, gene rearrangements, TMB, and MSI.

SNVs and indels

One benefit of target enrichment chemistry is the use of probes designed large enough to impart high binding specificity, but also allow hybridization to targets containing small mutations. As SNVs have been associated with cancer susceptibility in various cancer types, it is critical that any CGP method can detect these variants at low levels. TruSight Oncology 500 ctDNA v2 reproducibly detects SNVs and indels present at levels as low as 0.2% or 0.5% VAF, respectively (Figure 5 and Figure 6).

CNVs

Copy-number changes in several genes and tumor types have been associated with tumorigenesis.[27] TruSight Oncology 500 ctDNA v2 includes analysis of 59 CNV-associated genes and can call amplifications with a limit of detection at ≥ 1.3 fold for amplifications and ≤ 0.6 for deletions (Table 7).

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

§ Velsera was previously known as Pierian.

Figure 5: High analytical performance for key SNVs at LOD (0.2% VAF)—Synthetic control samples with known VAF for each single nucleotide variant were diluted to values ranging from 0.20%–0.50% VAF and analyzed using TruSight Oncology 500 ctDNA v2. SNVs present at levels at low as 0.2% were detectable.

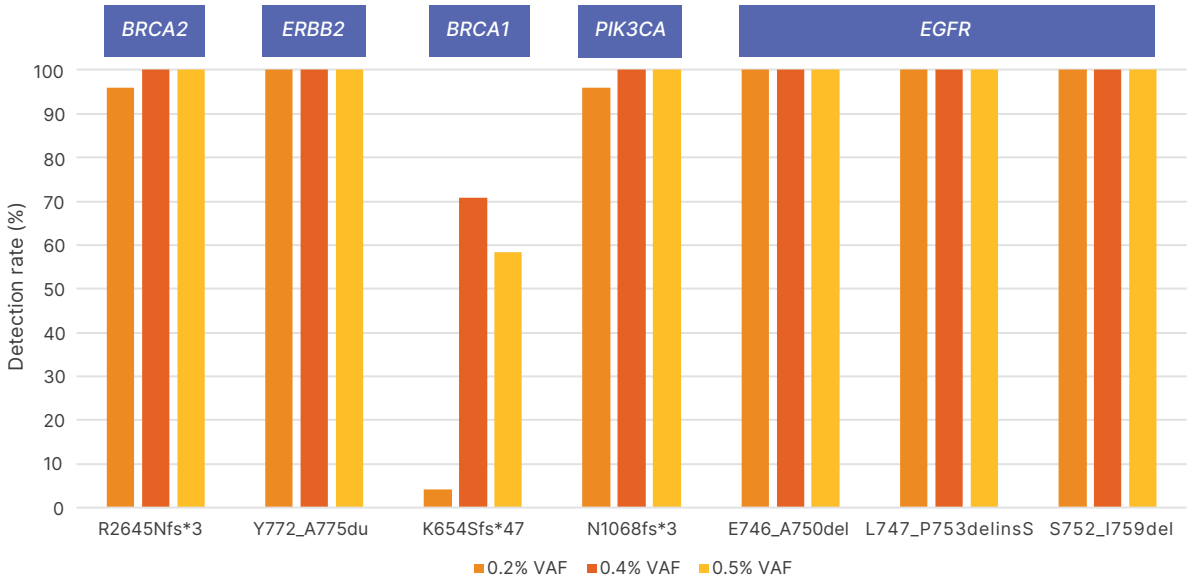

Figure 6: High analytical performance for indels at LOD (0.5% VAF)—Synthetic control samples with known VAF for each insertion or deletion were diluted to values ranging from 0.20%–0.50% VAF and analyzed by TruSight Oncology 500 ctDNA v2. *BRCA1* detection was lower due to the variant being in a highly homopolymer region, resulting in a high level of background noise.

Table 7: TruSight Oncology 500 ctDNA v2 analytical performance for CNVs

Gene	Expected fold-change	Observed fold-change	Detection rate
Amplifications			
ERBB2	1.5	1.50	100%
MET	1.5	1.55	100%
MYC	1.5	1.27	100%
ERBB2	1.4	1.73	100%
MET	1.4	1.46	100%
MYC	1.4	1.22	100%
ERBB2	1.3	1.35	100%
MET	1.3	1.38	100%
MYC	1.3	1.19	8%
ERBB2	1.2	1.19	100%
MET	1.2	1.22	100%
MYC	1.2	N/A	0
Deletions			
BRCA1	0.85	0.86	16%
BRCA2	0.85	N/A	0
BRCA1	0.80	0.79	100%
BRCA2	0.80	0.80	100%
BRCA1	0.70	0.69	100%
BRCA2	0.70	0.69	100%

Samples with known fold changes for gene amplifications using synthetic controls and cell lines for deletions were evaluated using TruSight Oncology 500 ctDNA v2. CNVs were diluted to three VAF levels. LOD ≥ 1.3 fold change for gene amplifications ≤ 0.6 for deletions. Note the strong correlation between expected and observed fold changes.

Gene rearrangements

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. TruSight Oncology 500 ctDNA v2 detects and characterizes gene rearrangements agnostic from the partner, even when present at low concentrations (Table 8).

Table 8: TruSight Oncology 500 ctDNA v2 analytical performance for gene rearrangements

Fusion	Expected VAF	Observed VAF	Detection rate
ALK:EML4	0.60%	0.48%	100%
GOPC;ROS1:CD74	0.60%	0.39%	100%
RET:NCOA4	0.60%	0.31%	100%
ALK:EML4	0.50%	0.43%	100%
GOPC;ROS1:CD74	0.50%	0.33%	100%
RET:NCOA4	0.50%	0.27%	100%
ALK:EML4	0.40%	0.36%	100%
GOPC;ROS1:CD74	0.40%	0.24%	100%
RET:NCOA4	0.40%	0.19%	100%
ALK:EML4	0.20%	0.18%	88%
GOPC;ROS1:CD74	0.20%	0.11%	100%
RET:NCOA4	0.20%	0.12%	83%

Samples with three known DNA fusions diluted to VAF levels ranging from 0.2% to 0.6% were evaluated with TruSight Oncology 500 ctDNA v2. The LOD for gene rearrangements = 0.5%. NGS-based assessment using TruSight Oncology 500 ctDNA v2 interrogates > 2300 homopolymer sites sized 6–7 bp, which helps reduce error rates and decrease potential false positives commonly found in homopolymer sequencing. VAF, variant allele frequency

IO gene signatures: MSI and TMB

MSI and TMB detection relies upon analysis of multiple genomic loci. NGS-based assessment with the TruSight Oncology 500 ctDNA v2 interrogates > 2300 homopolymer sites sized 6–7 bp, which helps reduce error rates and decrease potential false positives commonly found in homopolymer sequencing.[28] Featuring sensitive library preparation chemistry combined with advanced bioinformatics, TruSight Oncology 500 ctDNA v2 provides MSI detection achieved down to 0.3% tumor fraction (Figure 7).

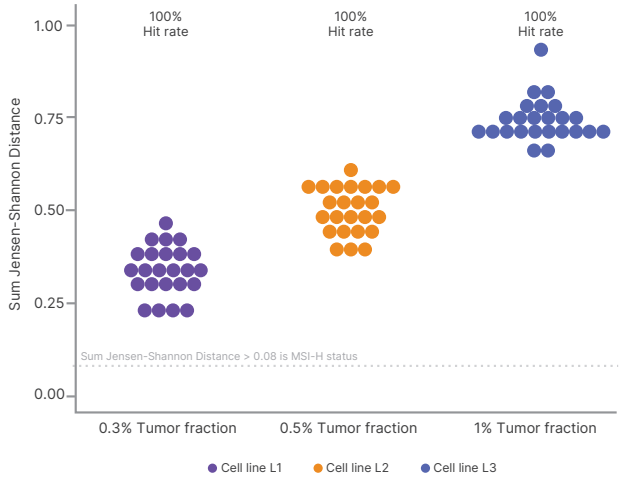

Figure 7: Sensitive MSI performance for IO research—Tumor fractions produced by titrating nucleosomal prepared cell lines with previously known MSI-H scores titrated into wildtype cell background. High MSI analytical sensitivity is achieved with proprietary DRAGEN TruSight Oncology 500 ctDNA v2.1 Analysis Software. More than 2300 homopolymer sites were assessed.

Obtaining a precise and reproducible bTMB value at low mutation levels can be challenging with smaller panels.[7] TruSight Oncology 500 ctDNA v2 combines comprehensive genomic content with a 1.94 Mb panel and sophisticated informatics algorithms to provide accurate bTMB estimations. The proprietary DRAGEN TruSight Oncology 500 ctDNA bioinformatics pipeline applies advanced filtering for both germline and clonal hematopoesis variants, resulting in a highly concordant tumor-only and tumor–normal workflows (R^2 = 0.992) (Figure 8).[28]

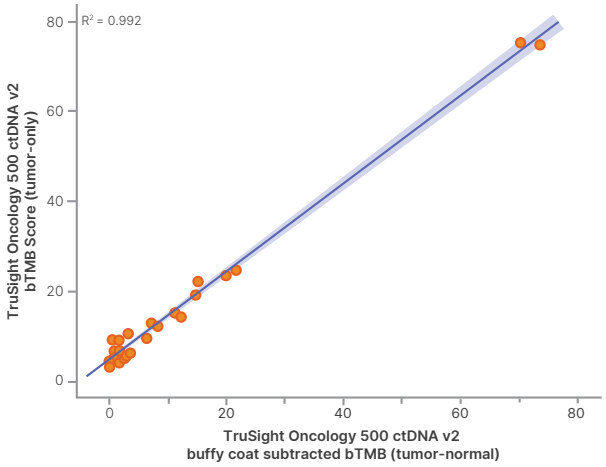

Figure 8: High bTMB data correlation between tumor-only and tumor-normal analysis workflows—Tumor-only bTMB scores produced with TruSight Oncology 500 ctDNA v2 with advanced bioinformatics and a panel large enough to detect TMB (> 1 Mb) show high concordance to bTMB scores produced from a paired tumor–normal workflow using plasma and buffy coat cfDNA.

Enhanced product attributes

Illumina offers high levels of service and support to ensure operational success for laboratories. To enable greater efficiency, TruSight Oncology 500 ctDNA v2 features:

- Advanced change notification—Illumina notifies laboratories six months before any significant changes are made to TruSight Oncology 500 ctDNA
- Certificate of Analysis¶—Every TruSight Oncology 500 ctDNA v2 is issued with a certificate of analysis (CoA) by the Illumina Quality Assurance Department that ascertains the product has met its predetermined product release specifications and quality
- Extended shelf life—The minimum guaranteed shelf life for TruSight Oncology 500 ctDNA v2 reagents is extended to six months, reducing the risk of product expiration and enabling labs to use reagents according to current testing needs

¶ CoA available in 2024.

Integrated solution enabling CGP from liquid biopsy

TruSight Oncology 500 ctDNA v2 is an NGS-based, multiplex research assay that analyzes hundreds of cancer-related biomarkers aligned with current guidelines and research from clinical trials from plasma samples simultaneously. The comprehensive assay detects multiple variant types in blood from 523 genes implicated in various tumor types and assesses IO and emerging biomarkers (bTMB, bMSI, *NTRK*, and *ROS1*), without requiring multiple samples for iterative testing.

Chemistry improvements have decreased the overall turnaround time to < 4 days, reduced the input requirement to 20 ng cfDNA, and lowered the limit of detection to 0.2% VAF (for SNVs). In addition, the automation-enabled workflow** reduces hands-on time and minimizes the burden on lab personnel, creating a streamlined lab for greater efficiency. Taking advantage of extensive genomic content, industry-leading sequencing technology, and enhanced software, TruSight Oncology 500 ctDNA v2 provides an integrated solution that enables CGP-based clinical research projects with minimal operational and analysis complexity.

Learn more

TruSight Oncology 500 ctDNA v2

NovaSeq 6000 System

DRAGEN secondary analysis

Illumina Connected Analytics

Illumina Connected Insights

Ordering information—Library preparation kits (manual)

Product	Catalog no.
TruSight Oncology 500 ctDNA v2 (24 samples)	20105899
TruSight Oncology 500 ctDNA v2 for use with NovaSeq 6000 S2 (24 samples)	20105901
TruSight Oncology 500 ctDNA v2 for use with NovaSeq 6000 S4 (24 samples)	20105902
TruSight Oncology 500 ctDNA v2 (24 samples) plus Velsera Interpretation Report	20105905
TruSight Oncology 500 ctDNA v2 plus Velsera Interpretation Report, for use with NovaSeq 6000 S2 (24 samples)	20105907
TruSight Oncology 500 ctDNA v2 plus Velsera Interpretation Report, for use with NovaSeq 6000 S4 (24 samples)	20105908
TruSight Oncology 500 ctDNA v2 (24 samples) plus Connected Insights Interpretation Report	Coming soon
TruSight Oncology 500 ctDNA v2 plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S2 (24 samples)	Coming soon
TruSight Oncology 500 ctDNA v2 plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S4 (24 samples)	Coming soon

** Automation-enabled workflow available in 2024.

Ordering information—Library preparation kits (automated)

Product	Catalog no.
TruSight Oncology 500 ctDNA v2 for Automation (48 samples)	20105900
TruSight Oncology 500 ctDNA v2 Automation Kit, for use with NovaSeq 6000 S2 (48 samples)	20105903
TruSight Oncology 500 ctDNA v2 Automation Kit, for use with NovaSeq 6000 S4 (48 samples)	20105904
TruSight Oncology 500 ctDNA v2 for Automation (48 samples) plus Velsera Interpretation Report	20105906
TruSight Oncology 500 ctDNA v2 Automation Kit plus Velsera Interpretation Report, for use with NovaSeq 6000 S2 (48 samples)	20105909
TruSight Oncology 500 ctDNA v2 Automation Kit plus Velsera Interpretation Report, for use with NovaSeq 6000 S4 (48 samples)	20105910
TruSight Oncology 500 ctDNA v2 for Automation (48 samples) plus Connected Insights Interpretation Report	Coming soon
TruSight Oncology 500 ctDNA v2 Automation Kit plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S2 (48 samples)	Coming soon
TruSight Oncology 500 ctDNA v2 Automation Kit plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S4 (48 samples)	Coming soon

Ordering information—Index adapters

Product	Catalog no.
IDT for Illumina UMI DNA/RNA UD Indexes Set A, Ligation (96 Indexes, 96 Samples)	20034701
IDT for Illumina UMI DNA/RNA UD Indexes Set B, Ligation (96 Indexes, 96 Samples)	20034702
IDT for Illumina UMI DNA/DNA Index Anchors Set A for Automation	20066404
IDT for Illumina UMI DNA/DNA Index Anchors Set B for Automation	20063213

Ordering information—Sequencing reagents

Product	Catalog no.
NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles)	20028314
NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles)	20028312

Ordering information—Analysis

Product	Catalog no.
Local variant reporting	
Illumina DRAGEN Server v4	20051343
Illumina DRAGEN Server Installation	20031995
Illumina DRAGEN Server v4 Support Plan	20085832
Field Delivered Applications Training	15032919
Cloud-based variant reporting	
ICA Basic Annual Subscription	20044874
ICA Professional Annual Subscription	20044876
ICA Enterprise Annual Subscription	20038994
ICA Enterprise Compliance Add-on	20066830
Subscription ICA Training and Onboarding	20049422
Variant interpretation	
Illumina Connected Insights – Annual Subscription	20090137
Illumina Connected Insights-Research – Annual Subscription	20112516
Illumina Connected Insights – Oncology Genome Equivalent Samples (VCF)	20090138
Illumina Connected Insights Training – Remote	20092376
Informatics Professional Services	20071787
Cloud storage	
Illumina Analytics – 1 iCredit	20042038
Illumina Analytics Starter Package – 1,000 iCredits	20042039
Illumina Analytics – 5,000 iCredits	20042040
Illumina Analytics – 50,000 iCredits	20042041
Illumina Analytics – 100,000 iCredits	20042042

References

1. Lim C, Tsao MS, Le LW, et al. Biomarker testing and time to treatment decision in patients with advanced nonsmall-cell lung cancer. *Ann Oncol*. 2015;26(7):1415-1421. doi:10.1093/annonc/mdv208
2. Yu TM, Morrison C, Gold EJ, Tradonsky A, Layton AJ. Multiple Biomarker Testing Tissue Consumption and Completion Rates With Single-gene Tests and Investigational Use of Oncomine Dx Target Test for Advanced Non-Small-cell Lung Cancer: A Single-center Analysis. *Clin Lung Cancer*. 2019;20(1):20-29.e8. doi:10.1016/j.cllc.2018.08.010
3. Reitsma M, Fox J, Borre PV, et al. Effect of a Collaboration Between a Health Plan, Oncology Practice, and Comprehensive Genomic Profiling Company from the Payer Perspective. *J Manag Care Spec Pharm*. 2019;25(5):601-611. doi:10.18553/jmcp.2019.18309
4. Zehir A, Benayed R, Shah RH, et al. Mutational landscape of metastatic cancer revealed from prospective clinical sequencing of 10,000 patients [published correction appears in Nat Med. 2017 Aug 4;23 (8):1004]. *Nat Med*. 2017;23(6):703-713. doi:10.1038/nm.4333
5. Kopetz S, Mills Shaw KR, Lee JJ, et al. Use of a Targeted Exome Next-Generation Sequencing Panel Offers Therapeutic Opportunity and Clinical Benefit in a Subset of Patients With Advanced Cancers. *JCO Precis Oncol*. 2019;3:PO.18.00213. Published 2019 Mar 8. doi:10.1200/PO.18.00213
6. Drilon A, Wang L, Arcila ME, et al. Broad, Hybrid Capture-Based Next-Generation Sequencing Identifies Actionable Genomic Alterations in Lung Adenocarcinomas Otherwise Negative for Such Alterations by Other Genomic Testing Approaches. *Clin Cancer Res*. 2015;21(16):3631-3639. doi:10.1158/1078-0432.CCR-14-2683
7. Buchhalter I, Rempel E, Endris V, et al. Size matters: Dissecting key parameters for panel-based tumor mutational burden analysis. *Int J Cancer*. 2019;144(4):848-858. doi:10.1002/ijc.31878
8. Chalmers ZR, Connelly CF, Fabrizio D, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9(1):34. Published 2017 Apr 19. doi:10.1186/s13073-017-0424-2
9. Hagemann IS, Devarakonda S, Lockwood CM, et al. Clinical next-generation sequencing in patients with non-small cell lung cancer. *Cancer*. 2015;121(4):631-639. doi:10.1002/cncr.29089
10. Rapisuwon S, Vietsch EE, Wellstein A. Circulating biomarkers to monitor cancer progression and treatment. *Comput Struct Biotechnol J*. 2016;14:211-222. Published 2016 Jun 1. doi:10.1016/j.csbj.2016.05.004

11. Aggarwal C, Thompson JC, Black TA, et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. *JAMA Oncol*. 2019;5(2):173-180. doi:10.1001/jamaoncol.2018.4305
12. Leighl NB, Page RD, Raymond VM, et al. Clinical Utility of Comprehensive Cell-free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-small Cell Lung Cancer. *Clin Cancer Res*. 2019;25(15):4691-4700. doi:10.1158/1078-0432.CCR-19-0624
13. Palmero R, Taus A, Viteri S, et al. Biomarker Discovery and Outcomes for Comprehensive Cell-Free Circulating Tumor DNA Versus Standard-of-Care Tissue Testing in Advanced Non-Small Cell Lung Cancer. *JCO Precision Oncology*. 2021;5:93-102. doi:10.1200/PO.20.00241
14. Rolfo C, Mack P, Scagliotti GV, et al. Liquid Biopsy for Advanced NSCLC: A Consensus Statement From the International Association for the Study of Lung Cancer. *J Thorac Oncol*. 2021;16(10):1647-1662. doi:10.1016/j.jtho.2021.06.017
15. Mosele F, Remon J, Mateo J, et al. Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2020;31(11):1491-1505. doi:10.1016/j.annonc.2020.07.014
16. Pascual J, Attard G, Bidard FC, et al. ESMO recommendations on the use of circulating tumour DNA assays for patients with cancer: a report from the ESMO Precision Medicine Working Group. *Ann Oncol*. 2022;33(8):750-768. doi:10.1016/j.annonc.2022.05.520
17. Illumina. TruSight Oncology 500 ctDNA data sheet. illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/trusight-oncology-500-ctdna-data-sheet-m-gl-00843/trusight-oncology-500-ctdna-data-sheet-m-gl-00843.pdf. Accessed September 20, 2023.
18. Bettegowda C, Sausen M, Leary RJ, et al. Detection of circulating tumor DNA in early- and late-stage human malignancies. *Sci Transl Med*. 2014;6(224):224ra24. doi:10.1126/scitranslmed.3007094
19. Mack PC, Banks KC, Espenschied CR, et al. Spectrum of driver mutations and clinical impact of circulating tumor DNA analysis in non-small cell lung cancer: Analysis of over 8000 cases. *Cancer*. 2020;126(14):3219-3228. doi:10.1002/cncr.32876
20. Saarenheimo J, Eigeliene N, Andersen H, Tiirola M, Jekunen A. The Value of Liquid Biopsies for Guiding Therapy Decisions in Non-small Cell Lung Cancer. *Front Oncol*. 2019;9:129. doi:10.3389/fonc.2019.00129
21. Roche. AVENIO ctDNA Analysis Kits. Sequencing. sequencing.roche.com/us/en/products/product-category/avenio-ctdna-analysis-kits.html. Accessed September 20, 2023.
22. Tempus. Tempus xF. www.tempus.com/oncology/genomic-profiling/xf/. Accessed September 20, 2023.
23. Personal Genome Diagnostics. PGDx elio plasma complete. https://www.personalgenome.com/products/elio-plasma-complete. Accessed September 20, 2023.
24. Illumina. TruSight Oncology UMI Reagents data sheet. illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-umi-reagents-datasheet-1000000050425.pdf. Accessed September 20, 2023.
25. Illumina. Sequencing accuracy with Unique Molecular Identifiers. illumina.com/techniques/sequencing/ngs-library-prep/multiplexing/unique-molecular-identifiers.html. Accessed September 20, 2023.
26. Socea JN, Stone VN, Qian X, Gibbs PL, Levinson KJ. Implementing laboratory automation for next-generation sequencing: benefits and challenges for library preparation. *Front Public Health*. 2023;11:1195581. doi:10.3389/fpubh.2023.1195581
27. Beroukhim R, Mermel CH, Porter D, et al. The landscape of somatic copy-number alteration across human cancers. *Nature*. 2010;463(7283):899-905. doi:10.1038/nature08822
28. Illumina. Analysis of TMB and MSI status with TruSight Oncology 500 application note. illumina.com/content/dam/illumina-marketing/documents/products/appnotes/trusight-oncology-500-tmb-analysis-1170-2018-009.pdf. Accessed September 20, 2023.

illumina®

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

M-GL-02196 v2.0