DATA SHEET

TruSight™ Oncology 500 ctDNA

Enabling comprehensive genomic profiling from liquid biopsy samples for solid tumor research

- Leverage minimally invasive blood samples as a complement to tissue biopsy or as an alternative when tissue is not readily available
- Assay DNA biomarkers across > 500 genes plus immuno-oncology signatures such as TMB and MSI
- Realize low limits of detection with UMI-based hybrid–capture library preparation and deep sequencing on the NovaSeq™ 6000 System
- Go from cfDNA to report interpretation in five days with a proprietary DRAGEN™ pipeline and multiple options for tertiary analysis and reporting

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Introduction

Liquid biopsy enables comprehensive analysis of circulating cell-free DNA (cfDNA) in plasma, providing a noninvasive approach for profiling solid tumors. To take advantage of liquid biopsy, it is critical to use a highly sensitive and specific analytical assay capable of detecting somatic mutations at low frequencies. TruSight Oncology 500 ctDNA harnesses the power of proven Illumina next-generation sequencing (NGS) technology to achieve this high analytical sensitivity and enables comprehensive genomic profiling of circulating tumor DNA (ctDNA) found in cfDNA (Figure 1, Table 1). Combining this advanced research solution with the bioinformatics power of the DRAGEN TruSight Oncology 500 ctDNA Analysis Software gives clinical researchers a DNA-to-report solution for evaluating multiple variant types across hundreds of genes in a single assay (Figure 2).

TruSight Oncology 500 ctDNA is compatible with NovaSeq 6000 v1.5 sequencing reagents. In addition to increases in operating efficiencies that result in potential price per sample reductions > 35%, these reagents offer an extended shelf-life of six months and improved Q30 scores.[1]

The power of liquid biopsy

Unlike a tissue biopsy that provides information from only a fraction of the tumor, liquid biopsy provides insights about intra- and inter-tumor heterogeneity throughout the body. Studies show that cfDNA analysis detects a significant number of guideline-recommended biomarkers and resistance alterations not found in matched tissue biopsies.[2] In addition, a non-small cell lung cancer study revealed that cfDNA analyses are highly concordant with tissue-based analyses.[3]

A foundation of comprehensive content

Content for TruSight Oncology 500 ctDNA was designed with recognized authorities in the oncology community. It 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 fusions and includes 523 genes for detecting variants likely to play a role in tumorigenesis. Biomarkers comprise small variants (SVs), insertions/deletions (indels), copy-number variants (CNVs), gene fusions, and complex immuno-oncology genomic signatures, such as microsatellite instability (MSI) and tumor mutational burden (TMB) (Table 2).

For panel content, view the TruSight Oncology 500 ctDNA Gene List.

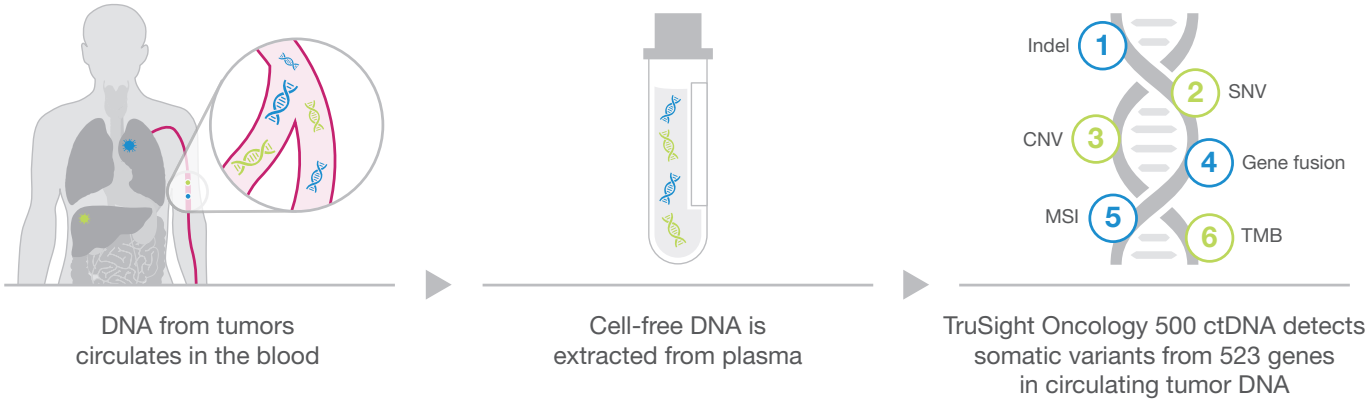

Figure 1: Liquid biopsy enables profiling of biomarkers for multiple variant types and multiple cancer types—Sophisticated variant calling algorithms and high depth of sequencing enable detection of key biomarkers in cfDNA with 0.5% limit of detection (LOD).

Table 1: TruSight Oncology 500 ctDNA at a glance

Parameter	TruSight Oncology 500 ctDNA
System	NovaSeq 6000 System
Panel size	1.94 Mb DNA
Panel content	523 genes 59 genes for CNVs 23 genes for gene fusions MSI (> 2400 loci) TMB
DNA input requirement	30 ng cfDNA[a]
Sample type	cfDNA derived from blood
Total assay time	5 days from library prep to variant report
Sequence run time	36 hr run, 10 hr analysis (S2 flow cell) 45 hr run, 22 hr analysis (S4 flow cell)
Sequence run	2 × 151 bp
Software version	DRAGEN TruSight Oncology ctDNA v2.5
Sample throughput	8 samples per run (S2 flow cell) 24 samples per run (S4 flow cell) 48 samples per library prep kit
Limit of detection	0.5% VAF for small variants ≥ 1.4-fold change for gene amplifications ≤ 0.6-fold change for gene deletions ≥ 2% tumor fraction for MSI
Analytical sensitivity	≥ 95% (at LOD for all variant types)
Analytical specificity	≥ 95%

a. Recommend quantification with Agilent TapeStation or Fragment Analyzer systems.

Table 2: Examples of variants detected using TruSight Oncology 500 ctDNA

Variant type	Relevant examples
SNVs and indels	*EGFR, POLE, TMPRSS2, BRAF*
Gene fusions	*ALK, ROS1, NTRK, RET*
CNVs	*HER2*
MSI	MSI-Score
TMB	TMB-Score

For a complete list of genes, visit illumina.com/products/by-type/clinical-research-products/trusight-oncology-500-ctdna.html

Proven technology for detecting low-level biomarkers

Using proven Illumina sequencing by synthesis (SBS) chemistry, TruSight Oncology 500 ctDNA enables comprehensive genomic profiling from just 30 ng cfDNA, making it an ideal alternative for use when tissue is not readily available or as a complement to tissue analysis. Library preparation takes advantage of target enrichment, using biotinylated probes and streptavidin-coated magnetic beads to enrich for selected targets from DNA-based libraries. Targeted hybridization–capture enrichment uses probes that are large enough to impart high binding specificity, but still allow hybridization to targets containing small mutations. This approach reduces sample dropouts in the presence of both natural allelic variations and sequence artifacts.

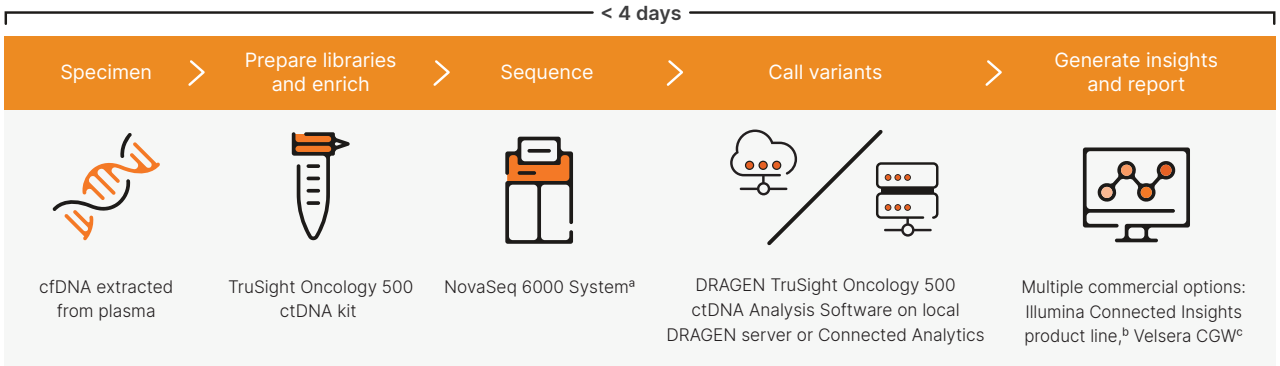

Figure 2: TruSight Oncology 500 ctDNA assay workflow—TruSight Oncology 500 ctDNA assay integrates into current lab workflows, going from cfDNA to a variant report in five days. a.NovaSeq 6000Dx System in RUO Mode has not been extensively tested, but is considered technically compatible; requires separate, stand-alone DRAGEN server if local secondary analysis is desired. 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. Other commercial options are available.

Because ctDNA represents a small fraction of cfDNA, powerful methods are required to separate signal from noise. Library preparation incorporates unique molecular identifiers (UMIs) that enable ultra-low frequency variant identification.[4] TruSight Oncology 500 ctDNA libraries are sequenced on the NovaSeq 6000 System at high depth (400M reads per sample at ~35,000×) to enhance sensitivity. The result is the ability to detect mutations at 0.5% variant allele frequency (VAF) for small variants, with 95% analytical sensitivity and > 99.995% analytical specificity (Table 3).

Table 3: Detection of low-level variants with high accuracy

Variant type	Analytical sensitivity[a]	Analytical specificity[b]
Small variants (≥ 0.5% VAF)	≥ 95%	≥ 99.995%
Gene amplifications (≥ 1.4-fold change)	≥ 95%	≥ 95%
Gene deletions (≤ 0.6-fold change)	≥ 95%	≥ 95%
Gene fusions (0.5%)	≥ 95%	≥ 95%
MSI high detection (≥ at 2% 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

DRAGEN TruSight Oncology 500 ctDNA Analysis Software uses accelerated, fully integrated bioinformatics algorithms to ensure optimal assay performance. The software performs 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. All pipeline components are within the DRAGEN platform, for additional performance improvements and efficiency.

Unlike qualitative results from PCR-based assays, DRAGEN TruSight Oncology 500 ctDNA Analysis Software provides a quantitative MSI score derived from > 2400 homopolymer MSI marker sites. For TMB analysis, the DRAGEN software optimizes sensitivity by measuring both nonsynonymous and synonymous SNVs and indels. After variant calling and error correction, the accuracy of TMB 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 Software runs locally on an Illumina DRAGEN Server v3 or v4 or in the cloud via Illumina Connected Analytics. The ultrarapid DRAGEN platform offers enhanced hardware and software that reduce data analysis time by ~85%, or from nine days to ~20 hours (Figure 3). With DRAGEN v3.10 compatibility, users can run TruSight Oncology ctDNA and TruSight Oncology 500 assays on the same local DRAGEN server, providing added flexibility. Connected Analytics offers a secure, streamlined, cloud-based genomics platform to scale up secondary analysis without the need to acquire and maintain more local infrastructure.[5] Further reducing manual touchpoints, Connected Analytics includes data streaming and autolaunch capabilities.

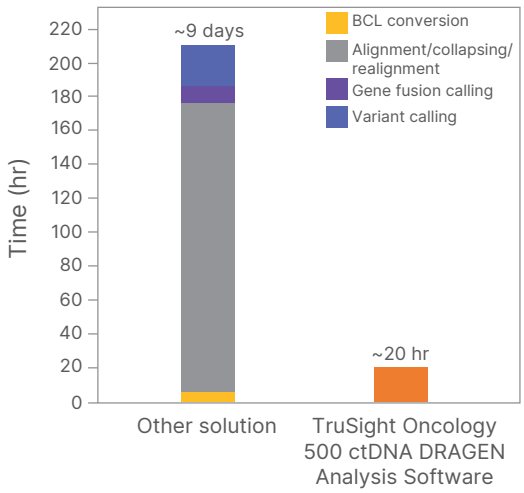

Figure 3: Onsite DRAGEN Server v4 reduces data analysis time—TruSight Oncology 500 ctDNA DRAGEN Analysis Software consolidates various data analysis steps into a single process that requires ~20 hrs, an ~85% reduction compared to an alternative solution to DRAGEN secondary analysis. Analysis times compare DRAGEN TruSight Oncology 500 ctDNA v2.1 pipeline on a DRAGEN Server v4 for 24 samples using an S4 flow cell to other solution using single node (128G memory, 24 cores CPU), nonparallelized pipeline for 24 samples using an S4 flow cell.

Learn more by reading the Security, privacy, and compliance with Illumina Connected Analytics technical note.

Variant insights and report generation are available through integration with Illumina Connected Insights and other commercial providers, including Velsera Clinical Genomics Workspace. 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.

Extensive validation delivers accurate and highly reproducible results

To demonstrate the high-quality results achieved with TruSight Oncology 500 ctDNA, Illumina performed various studies evaluating the ability to call SNVs, CNVs, gene fusions, TMB, and MSI (Figures 4 and 5, Tables 5 and 6).

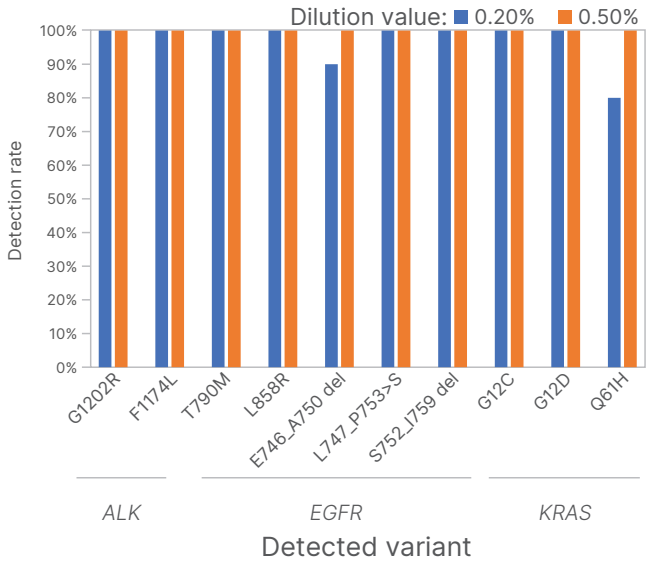

Figure 4: Small variant detection at low VAF—Samples with known VAF for each variant were diluted to values ranging from 0.10–1.00% VAF. Five replicates of each sample were analyzed with TruSight Oncology 500 ctDNA using 30 ng of commercial reference control DNA.

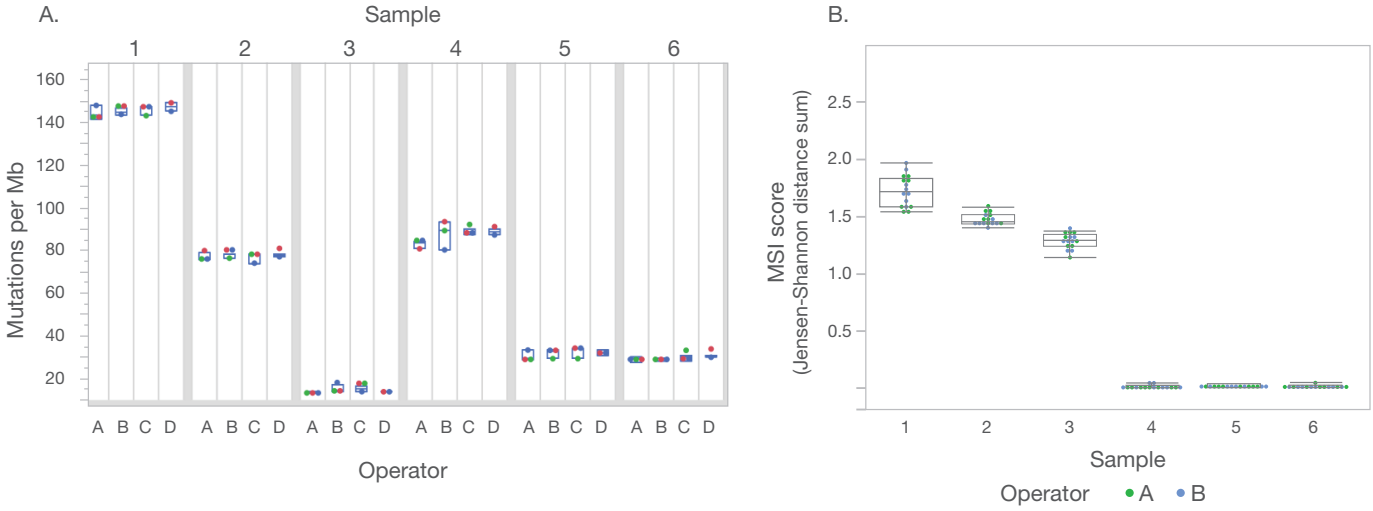

Figure 5: Reproducible TMB and MSI measurement—(A) TMB was evaluated in six different plasma samples (1–6) across four operators (A, B, C, D) in triplicate (green, blue, red dots). (B) MSI was evaluated in three nucleosomal prepped cell lines with known MSI-high status (samples 1–3) and three cfDNA samples from low prevalence MSI-high tumors (samples 4–6) across two different operators (A-green, B-blue).

Table 5: Sensitive detection of CNVs

Gene	Expected fold-change	Observed mean	Standard deviation	Detection rate
Amplifications				
BRCA2	1.8	1.4	0.01	100%
CCND3	1.5	1.3	0.01	100%
FGF14	1.3	1.5	0.01	100%
FGF3	1.6	1.4	0.01	100%
FGF4	1.7	1.4	0.01	100%
FGFR2	1.6	1.4	0.01	100%
MET	1.5	1.3	0.01	100%
MYC	1.9	1.7	0.02	100%
Deletions				
BRCA1	0.7	0.7	0	100%
BRCA2	0.6	0.6	0.01	100%

Samples with known fold-changes for gene amplifications and deletions were evaluated using TruSight Oncology 500 ctDNA with 30 ng of cfDNA input. Five replicates of each sample were analyzed.

Table 6: Gene fusion detection at low VAF

Gene fusion	Expected VAF	Observed VAF	Standard deviation	Detection rate
FGFR2-COL14A1	4.1%	4.1%	0.5%	100%
NPM1-ALK	3.4%	0.6%	0.1%	100%
FGFR3-BAIAP2L1	3.4%	0.8%	0.2%	100%
NPM1-ALK	2.4%	0.6%	0.1%	100%
EML4-ALK	1.7%	0.5%	0.1%	100%
CCDC6-RET	1.0%	0.7%	0.1%	100%
FGFR2-COL14A1	0.9%	0.4%	0.2%	100%
EML4-ALK	0.7%	0.2%	0.1%	100%
EML4-ALK	0.5%	0.5%	0.3%	100%
NPM1-ALK	0.5%	0.2%	0.0%	100%
NCOA4-RET	0.5%	0.2%	0.0%	100%
CCDC6-RET	0.2%	0.1%	0.1%	100%

Samples with known gene fusion allele frequencies ranging from ~0.5–4% were evaluated. Five replicates of each sample were analyzed using TruSight Oncology 500 ctDNA with 30 ng cfDNA input. Gene fusion directionality reported based on known expression. Consult the TruSight Oncology 500 ctDNA Local App User Guide for more information on DNA-based fusion directionality.

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 features:

- **Advanced change notification**—Illumina notifies laboratories six months before any significant changes are made to a product in the TruSight Oncology 500 portfolio.
- **Certificate of Analysis**—Every product in the TruSight Oncology 500 portfolio 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 reagents is extended to six months, reducing the risk of product expiration and enabling labs to use reagents according to current testing needs.

Summary

TruSight Oncology 500 ctDNA is an NGS-based, multiplex research assay that analyzes hundreds of cancer-related biomarkers from plasma simultaneously. Assay content is aligned with current guidelines and research from clinical trials. The single, comprehensive assay can detect multiple variant types from 523 genes implicated in various tumor types, without requiring multiple samples for iterative testing. TruSight Oncology 500 ctDNA also provides assessment of immuno-oncology and pan-cancer biomarkers (TMB, MSI, *NTRK*, *BRAF,* and *RET*). Taking advantage of extensive genomic content, industry-leading sequencing technology, and enhanced software, TruSight Oncology 500 ctDNA provides an integrated solution for accelerating clinical research projects with minimal operational and analysis complexity.

Learn more

TruSight Oncology 500 ctDNA

NovaSeq 6000 System

DRAGEN secondary analysis

Illumina Connected Analytics

Illumina Connected Insights

Ordering information

Product	Catalog no.
TruSight Oncology 500 ctDNA Kit (48 samples, 16 indexes)	20039252
TruSight Oncology 500 ctDNA Kit plus Pierian Interpretation Report (48 samples, 16 indexes)	20043410
Sequencing reagent kits	
NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles)	20028314
NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles)	20028312
NovaSeq Xp 4-Lane Kit v1.5	20043131
On-premise variant reporting	
Illumina DRAGEN Server v4	20051343
Illumina DRAGEN Server Advance Exchange Plan	20032797
Illumina DRAGEN Server Installation	20031995
Cloud-based variant calling	
Connected Analytics Basic Annual Subscription	20044874
Connected Analytics Professional Annual Subscription	20044876
Connected Analytics Enterprise Annual Subscription	20038994
Connected Analytics Enterprise Compliance Add-on (applies to Basic only)	20066830
Connected Analytics Training and Onboarding	20049422
Connected Analytics Data Storage: Illumina Analytics, 1 credit	20042038
Connected Analytics Data Storage: Illumina Analytics Starter Pack, 1000 credits	20042039
Connected Analtyics Data Storage: Illumina Analytics, 5000 credits	20042040
Connected Analytics Data Storage: Illumina Analytics, 50,000 credits	20042041
Connected Analytics Data Storage: Illumina Analytics, 100,000 credits	20042042

Ordering information, continued

Product	Catalog no.
Insights and report generation	
Illumina Connected Insights—Annual Subscription	20090137
Illumina Connected Insights—Research—Annual Subscription	20112516
Illumina Connected Insights—Oncology Genome Equivalent Sample-VCF	20090138
Illumina Connected Insights—Training—Remote	20092376
Informatics Professional Services	20071787

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