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TruSight™ Oncology 500 ctDNA*

A liquid biopsy assay that enables comprehensive genomic profiling, including TMB and MSI, from plasma.

- **Unlock immuno-oncology with TMB and MSI using a comprehensive genomic content assay**
  Same content as TruSight Oncology 500 for detection of SNVs, indels, CNVs, DNA fusions, TMB, and MSI from DNA
- **Achieve rapid, accurate variant detection**
  Highly sophisticated variant calling algorithm powered and accelerated by the DRAGEN Bio-IT Platform
- **Attain confidence in results**
  Hybrid-capture chemistry and UMI-based error correction improve variant calling and reduce artifacts
- **Leverage the power of the NovaSeq System**
  Enable comprehensive genomic profiling with the depth of sequencing required for cfDNA sensitivity

Multiple biomarkers and multiple tissues assessed through a single workflow

As molecular methods improve, the ability to use cell-free DNA (cfDNA) from plasma samples to analyze somatic variants from solid tumors has become possible. Next-generation sequencing (NGS) provides not only the sensitivity and accuracy required to detect low level variants in circulating tumor DNA (ctDNA), but also the ability to evaluate multiple variant classes across hundreds of genes in a single assay (Figure 1). Noninvasive plasma-based assays have emerged as an attractive complement to tissue-based assays, providing the opportunity to assess multiple samples over time, including tissues that are difficult to access.

With the optimization of NGS methods, noninvasive assays have the potential to detect mutations arising in new tissue locations and new locations in the genome. Recent studies in gastrointestinal cancer and non-small cell lung cancer revealed not only that cfDNA analyses are highly concordant with tissue-based analyses, but also that cfDNA analysis detected a significant number of guideline-recommended biomarkers and resistance alterations not found in matched tissue biopsies.[1,2]

From 30 ng of cfDNA, TruSight Oncology 500 ctDNA (Table 1) assesses multiple variant classes including single-nucleotide variants (SNVs), insertions/deletions (indels), copy-number variants (CNVs), DNA fusions, microsatellite instability (MSI), and tumor mutational burden (TMB) in a noninvasive assay (Table 2, Figure 1). TruSight Oncology 500 ctDNA utilizes the ultra-rapid DRAGEN™ Bio-IT Platform for data analysis, permitting the entire workflow to be completed in five days, from DNA library prep to consolidated variant reporting (Figure 2).

Table 1: TruSight Oncology 500 ctDNA product specifications

Parameter	Details
System	NovaSeq™ 6000 System,
Panel size	1.94 Mb DNA
Sample type	Plasma
DNA input requirement	30 ng cfDNA
Total assay time	5 days from library prep to variant report
Run time	36 hr run, 8 hrs analysis (S2 flowcell) 45 hr run, 22 hrs analysis (S4 flowcell)
Sample throughput	8 samples per run (S2 flowcell) 24 samples per run (S4 flowcell)
Sequence run	2 ×150 bp cycle, 800M paired-end reads
Kit size	48 samples

Comprehensive genomic profiling used in recent studies with large cohorts has shown that up to 90% of samples may have informative alterations.[3-8] With limited time to return results and limited access to tissues, a comprehensive assay assessing a wide range of biomarkers increases the chance of obtaining relevant information in a single sample. TruSight Oncology 500 ctDNA enables comprehensive genomic profiling from cfDNA. As a plasma based assay, TruSight Oncology 500 ctDNA can be used to profile resistance markers, support longitudinal monitoring, and identify relevant biomarkers when tissue testing is not optimal.

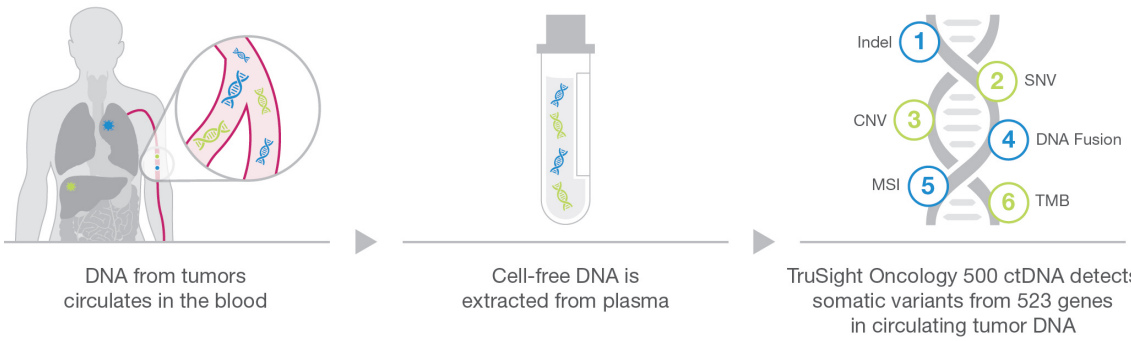

Figure 1: Liquid biopsy enables profiling of biomarkers for multiple variant types and multiple cancer types— Sophisticated error correction algorithms and high depth of sequencing enables detection of key biomarkers in cfDNA with limit of detection as low as 0.5% variant allele frequency.

* Under development, coming 2020

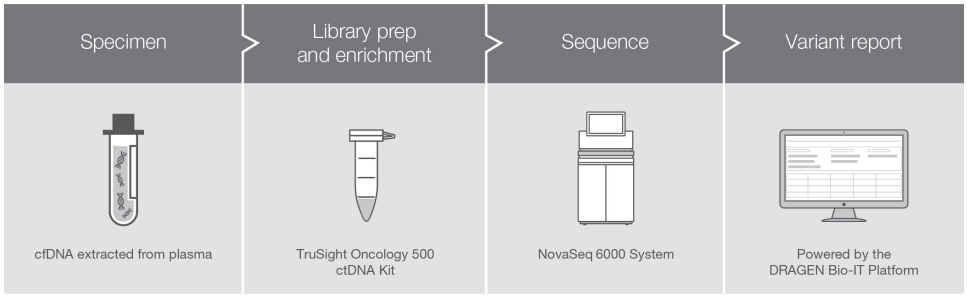

Figure 2: NGS workflow—The TruSight Oncology 500 ctDNA assay integrates into current lab workflows, going from nucleic acids to a variant calls in 5 days.

By harnessing expertise from recognized authorities in the oncology community, content was designed to include both current guidelines and emerging biomarkers, including comprehensive coverage of genes involved in key guidelines and clinical trials for multiple tumor types. Using proven Illumina technology for cfDNA analysis of variants likely to play a role in tumorigenesis, the comprehensive content of TruSight Oncology 500 ctDNA serves as a strong foundation for the development of future oncology diagnostic solutions.

Table 2: Variants detected by TruSight Oncology 500 ctDNA

Variant type	Relevant examples
SNVs and indels	*EGFR* exon 19, *BRAF* V600E, *EGFR* T790M
DNA fusions	*ALK*, *ROS1*, *NTRK*, *RET*
CNVs	*HER2*
MSI	MSI-High
TMB	TMB-High

Error correction enables accurate detection of low-level biomarkers

TruSight Oncology 500 ctDNA library preparation is based on proven target enrichment chemistry using biotinylated probes and streptavidin-coated magnetic beads to purify selected targets from DNA-based libraries. A benefit of target enrichment chemistry is the use of probes designed large enough to impart high binding specificity, but also allowing hybridization to targets containing small mutations. This mechanism reduces sample dropouts in the presence of both natural allelic variations and sequence artifacts.

Because ctDNA represents a small fraction of cell-free DNA (cfDNA), powerful methods are required to separate signal from noise. The TruSight Oncology 500 ctDNA assay implements higher depth of sequencing (> 35,000×) to enhance sensitivity. Library preparation also incorporates unique molecular identifiers (UMIs) that are used during data analysis to reduce inherent sequencing errors.[11] Maintaining high specificity (low false positives) is key in calling mutation at ultra-low frequency. UMI introduction enables detection of mutations at 0.5% variant allele frequency (VAF) for small variants, with 95% sensitivity and >99.995% specificity (Table 3). Data analysis software, which is run on the separately provided DRAGEN Bio-IT platform, implements sophisticated error correction prior to variant calling that was developed in concert with the assay reagents.

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

Variant type	Sensitivity	Specificity
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 rearrangements	≥ 95%	≥ 95%
MSI high detection (≥ at 2% tumor fraction)	≥ 95%	≥ 95%

To achieve > 95% sensitivity at 0.5% VAF, performance of TruSight Oncology 500 ctDNA was validated based on 35,000× sequence coverage with 30 ng cfDNA, an input amount based on expected yields from plasma samples. If higher yields of cfDNA are obtained, the assay can ideally be used with more input amounts. In cases where input is higher or lower than the recommended amount, sensitivity should theoretically increase or decrease accordingly (Table 4).

Table 4: Predicted sensitivity at varied input amounts and limits of detection.

Input (cfDNA)	Variant allele frequency								
	0.20%	0.30%	0.40%	0.50%	0.60%	0.70%	0.80%	0.90%	1.00%
10 ng	38.46	59.39	74.54	84.57	90.88	94.71	96.97	98.29	99.04
30 ng	90.83	98.27	99.70	99.95	99.99	100.00	100.00	100.00	100.00
50 ng	99.02	99.95	100.00	100.00	100.00	100.00	100.00	100.00	100.00
70 ng	99.91	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
100 ng	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

Calculations based upon 35,000x coverage for small hotspot variants. Hotspot variants occur > 50 in the COSMIC database.

Comprehensive content design

The TruSight Oncology 500 ctDNA panel includes a comprehensive list of biomarkers commonly mutated in numerous neoplasm types. Analysis of various types of biomarkers relevant to a given tumor type (SNVs, indels, DNA fusions, CNVs, TMB, MSI) can be assessed from the same sample in a single assay (Figure 1). The panel uses a probe design that enables capture of both known and novel gene fusions. The TruSight Oncology 500 ctDNA panel includes 523 genes for variant detection . For a complete list of genes, visit www.illumina.com/tso500-ctDNA.

Accelerated pipeline powered by DRAGEN Bio-It Platform

Enhanced hardware and software on the DRAGEN platform reduce time of data analysis time from nine days to ~ 20 hours (Table 5). Using a sophisticated set of proprietary algorithms, the DRAGEN Platform was optimized for use with the TruSight Oncology ctDNA assay to perform alignment, collapsing with error correction, and variant calling that includes TMB and MSI, from cfDNA. Unlike qualitative results from PCR and IHC-based MSI assays, TruSight Oncology 500 ctDNA provides a quantitative MSI score derived from over 70 homopolymer MSI marker sites. For TMB analysis, TruSight Oncology 500 ctDNA optimizes sensitivity by measuring both nonsynonymous and synonymous SNVs and indels. After variant calling and error correction, accuracy of TMB measurement is further enhanced by filtering germline variants, low-confidence variants, and variants associated with clonal hematopoesis of indeterminate potential.

Table 5: Time required for data analysis is dramatically reduced with the DRAGEN Bio-IT Platform

Data analysis step	Non-DRAGEN solution*	TruSight Oncology 500 ctDNA DRAGEN analysis solution
BCL conversion	6 hours	1 hour
Alignment + collapsing + realignment	170 hours	11 hours
Fusion calling	10 hours	2 hours
Variant calling	24 hours	8 hours
Total time	~9 days	~20 hours

* Single node, non-parallelized pipeline in server cluster

Summary

Labs can independently use the TruSight Oncology 500 ctDNA kit to enable comprehensive genomic profiling from plasma samples. Liquid biopsy tests can be used to overcome tissue limitations (limited tissue, tumor heterogeneity), profile resistance biomarkers, and assess longitudinal monitoring. Using hybrid-capture chemistry with sophisticated tools to reduce errors and high depth of sequencing, high-quality data is obtainable from samples with low limit of detection (0.5% VAF).

TruSight Oncology 500 ctDNA is an NGS-based, multiplex assay that analyzes hundreds of cancer-related biomarkers in a single assay. The assay content is aligned with current guidelines and clinical trials, with the ability to detect multiple variant types from 523 genes implicated in various tumor types, without requiring multiple samples for iterative testing. Taking advantage of extensive genomic content, TruSight Oncology 500 ctDNA also provides assessment of immunotherapy biomarkers (TMB and MSI). Leverage the power of TruSight Oncology 500 ctDNA to accelerate your research goals today.

Learn more

For more information about TruSight Oncology 500 ctDNA, visit www.illumina.com/tso500-ctDNA

Ordering information*

Library prep kit plus NovaSeq System reagents	No. of indexes/samples	Catalog no.
TruSight Oncology 500 ctDNA (48 Samples), For Use with NovaSeq 6000 (S2 Reagent Kit) (Includes DNA library prep and enrichment reagents. Includes NovaSeq core reagents)	16 indexes 48 samples	20039253
TruSight Oncology 500 ctDNA (48 Samples), For Use with NovaSeq 6000 (S4 Reagent Kit) (Includes DNA library prep and enrichment reagents. Includes XP 4-Lane Kits and NovaSeq core reagents)	16 indexes 48 samples	20039254

* Under development, available in 2020.

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