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TruSight™ Oncology 500 ctDNA

Enabling in-house comprehensive genomic profiling from liquid biopsy samples.

Highlights

- **Enable comprehensive genomic profiling from blood**
  Assess DNA variants across 523 genes in house, including bTMB and bMSI*
- **Unlock liquid biopsy in-house**
  Leverage noninvasive blood plasma samples as an alternative, or complement, to limited tissue samples
- **Produce trustworthy results**
  Realize low limits of detection with UMI-based hybrid–capture library preparation, high-intensity sequencing with the NovaSeq™ 6000 System, and advanced bioinformatics
- **Take advantage of an integrated workflow**
  Go from cfDNA to report interpretation using the DRAGEN™ Bio-IT Platform for variant calling in five days

* bTMB and bMSI refer to plasma-based (blood) markers

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 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 sensitivity and enables comprehensive genomic profiling of circulating tumor DNA (ctDNA) found in cfDNA (Figure 1, Table 1). Combining this advanced 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.

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.

Table 1: TruSight Oncology 500 ctDNA at a glance

Parameter	Details
Instrument	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
Sequencing run time	36 hr run, 10 hrs analysis (S2 flow cell) 45 hr run, 22 hrs analysis (S4 flow cell)
Sequence run	2 × 151 bp
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

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. Recent studies show that cfDNA analysis detected a significant number of guideline-recommended biomarkers and resistance alterations not found in matched tissue biopsies.[1] In addition, a non-small cell lung cancer study revealed that cfDNA analyses are highly concordant with tissue-based analyses.[2]

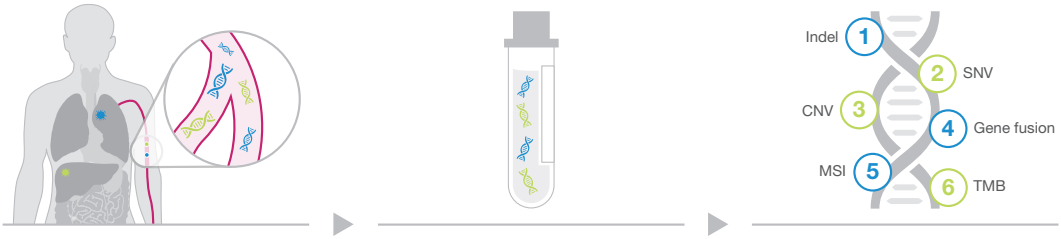

Figure 1: Liquid biopsy enables profiling of biomarkers for multiple variant types and multiple cancer types—Sophisticated variant calling algorithms and high depth of sequencing enable detection of key biomarkers in cfDNA with 0.5% limit of detection (LOD).

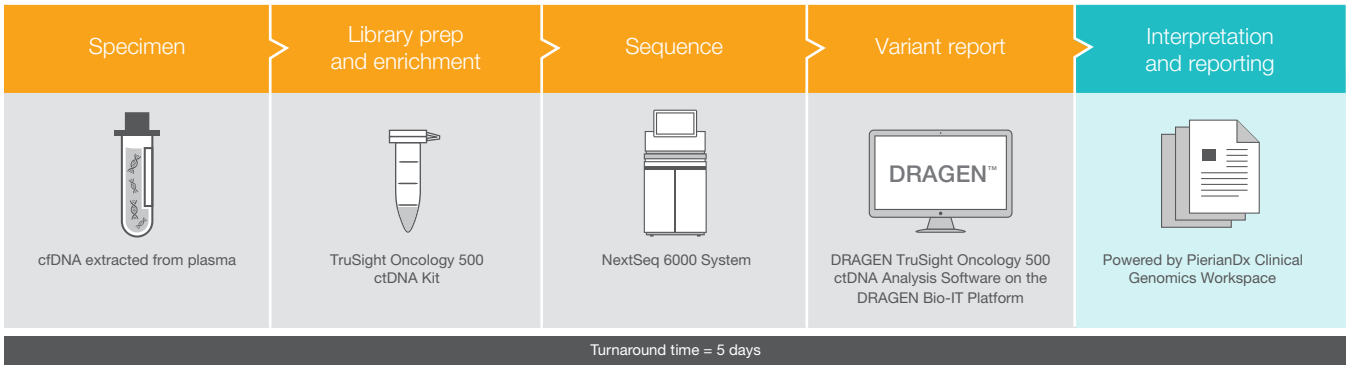

Figure 2: TruSight Oncology 500 ctDNA assay workflow—The TruSight Oncology 500 ctDNA assay integrates into current lab workflows, going from cfDNA to a variant report in five days.

A foundation of comprehensive content

Content for TruSight Oncology 500 ctDNA 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 fusions and includes 523 genes for detecting variants likely to play a role in tumorigenesis. Biomarkers comprise single-nucleotide variants (SNVs), 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 a complete list of genes, visit www.illumina.com/products/by-type/clinical-research-products/trusight-oncology-500-ctdna.html.

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

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 testing is not available. Library preparation takes advantage of target enrichment chemistry, using biotinylated probes and streptavidin-coated magnetic beads to enrich for selected targets from DNA-based libraries. Targeted hybridization–capture enrichment chemistry 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.

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.[3] 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 and accelerated analysis

DRAGEN TruSight Oncology 500 ctDNA Analysis Software uses accelerated, fully integrated bioinformatics algorithms to overcome cfDNA challenges and 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.

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 on a local Illumina DRAGEN Server v3.* This ultra-rapid platform offers enhanced hardware and software that reduce data analysis time by ~85%, or from nine days to ~20 hours (Table 4).

PierianDx Clinical Genomics Workspace completes tertiary analysis. Simply upload variant report files directly into the Clinical Genomics Workspace cloud. Clinical Genomics Workspace performs variant annotation and filtering for smooth interpretation and reporting. From thousands of variants in the genome, Clinical Genomics Workspace filters and prioritizes biologically relevant variants for the final automated, customizable genomic report. The entire workflow, from cfDNA to consolidated variant reporting, takes only five days (Figure 2).

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 3 and 4, Tables 5 and 6).

Table 4: Time required for data analysis is reduced with the onsite DRAGEN Server v3

Data analysis step	Non-DRAGEN solution[a]	TruSight Oncology 500 ctDNA DRAGEN Analysis Software
BCL conversion	6 hours	1 hour
Alignment + collapsing + realignment	170 hours	11 hours
Gene fusion calling	10 hours	2 hours
Variant calling	24 hours	8 hours
Total time	~9 days	~20 hours (~85% reduction)

a. Single node (128G memory, 24 cores CPU), nonparallelized pipeline for 24 samples using an S4 flow cell

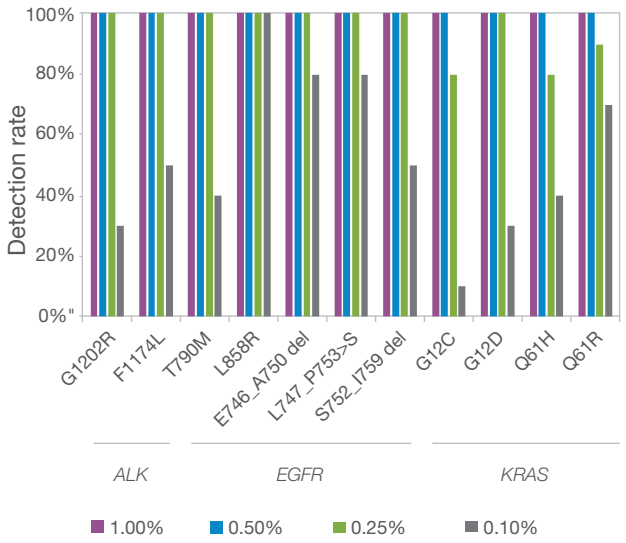

Figure 3: Small variant detection at low VAF—Samples with known VAF for each variant were diluted to values ranging from 0.10-1.00% VAF. Five replicates of each sample were analyzed with TruSight Oncology 500 ctDNA using 30 ng of commercial reference control DNA.

Summary

TruSight Oncology 500 ctDNA is an NGS-based, multiplex assay that analyzes hundreds of cancer-related biomarkers from plasma simultaneously. 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. TruSight Oncology 500 ctDNA also provides assessment of immuno-oncology and emerging biomarkers (TMB, MSI, *NTRK*, and *ROS1*). 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, in your own laboratory with minimal operational and analysis complexity.

* A cloud-based DRAGEN TruSight Oncology 500 ctDNA Analysis Software solution is coming soon.

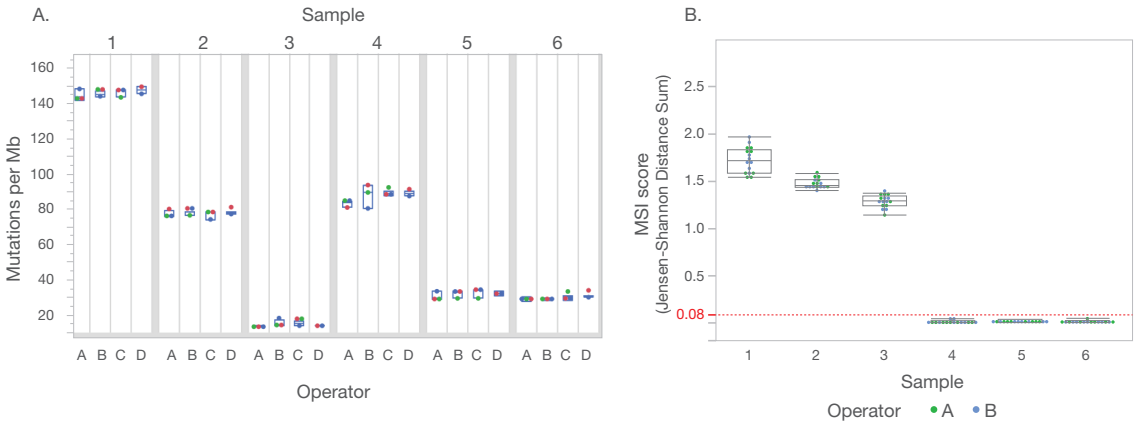

Figure 4: Reproducible TMB and MSI measurement—(A) TMB was evaluated in six different plasma samples across four operators in replicate. (B) MSI was evaluated in three nucleosomal prepped cell lines with known MSI-high status (1-3) and three cfDNA samples from low prevalence MSI-high tumors (4-6) across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

Table 5: Sensitive detection of CNVs

Gene	Expected fold-change	Observed mean	Standard deviation	Detection rate
Amplifications				
AKT2	1.4	1.4	0.02	100%
BRAF	1.5	1.5	0.01	100%
BRCA2	1.8	1.5	0.01	100%
CCND3	1.5	1.4	0.01	100%
CDK6	1.5	1.5	0.01	100%
FGF14	1.3	1.5	0.01	100%
FGF3	2.1	1.6	0.01	100%
FGF4	1.4	1.2	0.01	100%
FGFR2	1.3	1.5	0.01	100%
MET	1.4	1.5	0.02	100%
MYC	1.7	1.8	0.02	100%
Deletions				
BRCA1	0.8	0.8	0.01	100%
BRCA2	0.8	0.8	0.01	100%
AR	0.7	0.8	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%	4.2%	0.5%	100%
NPM1-ALK	3.4%	0.7%	0.2%	100%
FGFR3-BAIAP2L1	3.4%	0.7%	0.2%	100%
NPM1-ALK	2.4%	0.4%	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%	0.1%	100%
EML4-ALK	0.7%	0.2%	0.1%	100%
NCOA4-RET	0.5%	0.1%	0.0%	100%
EML4-ALK	0.5%	0.8%	0.2%	100%
NPM1-ALK	0.5%	0.1%	0.0%	100%
CCDC6-RET	0.2%	0.2%	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.

Learn more

To learn more about TruSight Oncology 500 ctDNA, visit www.illumina.com/tso500-ctDNA

To more about the DRAGEN Bio-IT Platform, visit www.illumina.com/DRAGEN

References

1. Parikh AR, Leshchiner I, Elagina L, et al. Liquid versus tissue biopsy for detecting acquired resistance and tumor heterogeneity in gastrointestinal cancers. *Nat Med*. 2019;25(9):1415-1421.
2. 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.
3. Illumina (2017) TruSight Oncology UMI Reagents. www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-umi-reagents-datasheet-1000000050425.pdf. Accessed May 4, 2020.

Ordering information

Product	Quantity	Catalog no.
TruSight Oncology 500 ctDNA Kit	48 samples/ 16 indexes	20039252
TruSight Oncology 500 ctDNA Kit plus PierianDx Interpretation Report	48 samples/ 16 indexes	20043410
NovaSeq 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	1 kit	20043131
On-premise variant reporting		
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 1	1-year license	20042100
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 2	1-year license	20042101
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 3	1-year license	20042102
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 4	1-year license	20042103
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 5	1-year license	20042104
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 6	1-year license	20042105
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 7	1-year license	20042106
DRAGEN TruSight Oncology 500 ctDNA Analysis Software, Level 8	1-year license	20042107
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Illumina DRAGEN Server Advance Exchange Plan		20032797
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Illumina • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

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