illumina®

DATA SHEET

TruSight™ Oncology 500 ctDNA v2

Enabling sensitive, fast CGP from liquid biopsy samples

Detect biomarkers present as low as 0.2% VAF from 20 ng ctDNA (5–30 ng possible)

Obtain comprehensive results in 2.5–4 days with manual or automated options

Analyze > 500 genes and immuno-oncology (IO) genomic signatures (bMSI, bTMB) in one assay

Leverage transformative economics and small batch sizes with the NextSeq™ 2000 System and the NovaSeq™ X Series

The value ctDNA and liquid biopsy bring to CGP

Understanding the genomic basis of cancer can help pinpoint alterations that fuel the disease, enabling biomarker discovery and advancements in precision medicine. Among the tools used in oncology research is comprehensive genomic profiling (CGP). Next-generation sequencing (NGS) can enhance CGP by facilitating the assessment of 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 variants of interest 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 discovered, including immuno-oncology (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 relies on solid tumor tissue samples, typically formalin-fixed, paraffin-embedded (FFPE) specimens. However, in up to 25% of cases, tissue is inadequate for testing, the tumor is inaccessible, or the process does not yield results within a useful timeframe. In these cases, performing CGP with circulating tumor DNA (ctDNA) from a blood-based liquid biopsy can provide insights into the genomic landscape of the tumor. Detection of ctDNA has been reported across all stages of cancer progression and in multiple solid tumor types, including lung, breast, colorectal, and ovarian cancers.[10]

Using ctDNA for CGP has several advantages:

- Access sample easily through a minimally invasive blood draw procedure[11]
- Capture clones from multiple tumors or even the same tumor,[12] overcoming inherent sampling bias present with solid tumor biopsy and expanding the ability to identify more alterations[12-15]
- Obtain temporal and spatial information about intra- and inter-tumor heterogeneity[11]
- Repeat analysis to assess clonal selection

Liquid biopsy provides a noninvasive approach to obtaining cell-free DNA (cfDNA), which includes ctDNA, from blood plasma for tumor profiling (Figure 1). For certain tumor types, such as non-small cell lung cancer (NSCLC), adding CGP from liquid biopsy to tissue testing can increase the identification of variants by 15–48%.[13,14,16] In addition, studies of NSCLC show high concordance between cfDNA- and tissue-based analyses.[14] Liquid biopsy is an active area of research, driven by the need for alternatives when tissue samples are limited or unavailable.[17-19]

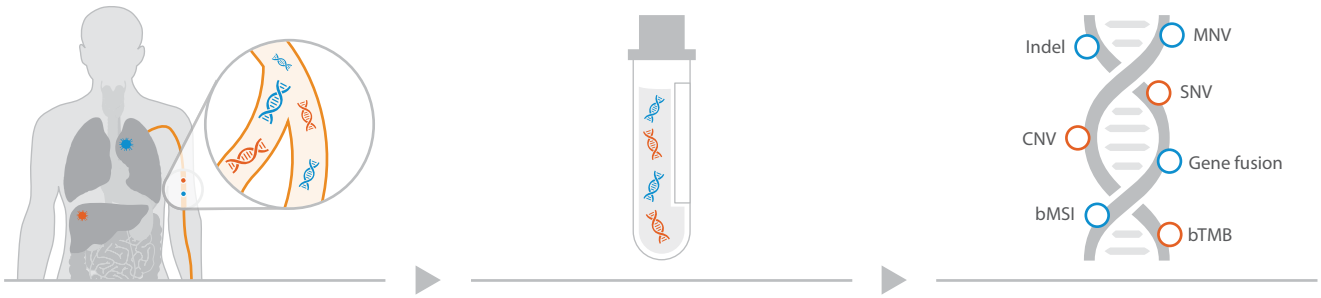

Figure 1: Liquid biopsy enables a noninvasive approach for CGP

A simple blood draw yields ctDNA from cell-free (cfDNA) in plasma, which is then analyzed using TruSight Oncology 500 ctDNA v2 to detect cancer-relevant biomarkers referenced in evidence-based guidelines.

bMSI, blood-based microsatellite instability; bTMB, blood-based tumor mutational burden; CGP, comprehensive genomic profiling; ctDNA, circulating tumor DNA.

TruSight Oncology 500 ctDNA v2

A robust liquid biopsy assay requires a highly sensitive and specific assay capable of detecting low-frequency somatic mutations in ctDNA. The original TruSight Oncology 500 ctDNA assay met this need by leveraging proven Illumina NGS technology to deliver analytical sensitivity.[20]

TruSight Oncology 500 ctDNA v2 incorporates advances in chemistry and workflow efficiency that improve assay performance and enable faster turnaround times (Table 1 and Table 2). The improved analytical performance supports reliable detection of low-frequency variants (Table 3). In addition, the assay is compatible with Illumina mid-throughput and high-capacity sequencing systems, enabling laboratories to select the configuration that best aligns with their study designs (Table 4).

Table 1: TruSight Oncology 500 ctDNA v2 advances

Benefit	TruSight Oncology 500 ctDNA v2	TruSight Oncology 500 ctDNA
Improved assay sensitivity	Separate end-repair and A-tailing steps provide cleaner, more efficient library preparation	Combined end-repair and A-tailing
More streamlined workflow and improved user experience	Plate-based indexes/UMIs reduce handling steps	Tube-based indexes/UMIs
Faster, single day workflow	Single hybridization-capture step	Two hybridization-capture steps
More scalability	192 indexes	16 indexes
Broader batch sizes	4–64 samples[a]	8–48 samples
Automation enabled	Yes	No

a. Batches of four samples are available on the NextSeq 2000 System, NovaSeq 6000 System, and NovaSeq X Series.
UMI, unique molecular identifier.

Table 2: TruSight Oncology 500 ctDNA v2 specifications

Parameter	TruSight Oncology 500 ctDNA v2
Automation capability	• Hamilton NGS STAR MOA • Beckman Coulter Life Sciences Biomek i7 • Hamilton NGS STARlet (coming soon)
Hands-on time	• 8–24 samples (manual): ~2.5 hr • 8–24 samples (automated): ~1.5 hr • 48 samples (manual): ~4.5 hr • 48 samples (automated): ~1.5 hr
Library preparation time[a]	• 8–24 samples (manual): ~8.5 hr • 8–24 samples (automated): ~9.5 hr • 48 samples (manual): ~10 hr • 48 samples (automated): ~11 hr
Library preparation	• 24-sample (manual) • 48-sample (automated)
Panel size	1.94 Mb DNA
Panel content	• 523 genes for small variants • 59 genes for CNVs • 23 genes for gene rearrangements • bMSI (> 2300 loci) • bTMB (> 1 Mb)
Sample type	cfDNA derived from blood plasma
DNA input	20 ng cfDNA (5–30 ng possible)[b]
Sequence run read length	2 × 151 bp
Sequence	35,000×
Secondary analysis time[c]	• 4 samples: 1 hr 45 min • 8 samples: 2 hr 15 min • 24 samples: 3 hr • 64 samples: 4 hr 10 min
Bioinformatics features	• SNV, MNV, indel, CNV, fusion calling, bMSI, and bTMB • Advanced UMI, noise-reduction, and error correction algorithms • Fragmentomics-based CHIP variant filtering • Germline variant filtering, including database- and proximity-based • Max Somatic VAF for tumor fraction estimation • Contamination detection optimized for rearranged genomes

a. Includes library preparation, enrichment, and bead-based normalization.
b. Recommend quantification with Agilent TapeStation or Fragment Analyzer systems. To learn more about input amounts, read the Using lower input amounts with TruSight Oncology ctDNA v2 technical note.
c. Times listed correspond to analysis time in Illumina Connected Analytics (cloud) and include 0.5 hr queuing time; queue times may vary.
CNV, copy number variation; bMSI, blood-based microsatellite instability; bTMB, blood-based tumor mutational burden; UMI, unique molecular identifiers; VAF, variant allele frequency.

Table 3: TruSight Oncology 500 ctDNA v2 assay performance[a]

Parameter	Analytical sensitivity[b]	Analytical specificity[c]
Small DNA variants • SNV ≥ 0.2% VAF (≥ 0.4% VAF) • SNV hotspots ≥ 0.2% VAF • MNV ≥ 0.5% VAF • Indels ≥ 0.5% VAF	≥ 90% (≥ 95%) ≥ 95% ≥ 90% ≥ 90%	≥ 99.999%
Gene amplifications ≥ 1.3-fold change	≥ 95%	≥ 95%
Gene deletions ≤ 0.6-fold change	≥ 95%	≥ 95%

a. Performance characteristics were measured on NovaSeq 6000 System using 20 ng of cfDNA and 35,000x coverage, as recommended. Equivalent performance was shown on the NextSeq 2000 System and NovaSeq X Series
b. Analytical sensitivity is defined as percent detection at the stated variant level.
c. Analytical specificity is defined as the ability to detect a known negative.

MNV, multinucleotide variant; SNV, single nucleotide variant; VAF, variant allele frequency.

Table 4: TruSight Oncology 500 ctDNA v2 sequencing system compatibility

Parameter	TruSight Oncology 500 ctDNA v2		
System	NextSeq 2000 System	NovaSeq 6000 System or 6000Dx Instrument[a]	NovaSeq X Series
No. samples per flow cell (flow cell)	4 (P4)	4 (S1) 8 (S2) 24 (S4)	4 (1.5B) 24 (10B) 64 (25B)
Sample throughput (per run)	4	4–48	4–128
Sequence run time	44 hr	25–44 hr	22–48 hr
Total assay time	3 days	2.5–3.5 days	2.5–4 days

a. In RUO mode.

Comprehensive content

The TruSight Oncology 500 ctDNA v2 content was designed with recognized authorities in the oncology community. It includes current and emerging biomarkers with comprehensive coverage of genes referenced in evidence-based 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 in the future (Appendix). Biomarkers include single nucleotide variants (SNVs), multinucleotide 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 5).

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

Table 5: 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*
bMSI	bMSI score
bTMB	bTMB score

bMSI, blood-based microsatellite instability; bTMB, blood-based tumor mutational burden; CNV, copy number variant; SNV, single nucleotide variant.

Fast, integrated workflow

TruSight Oncology 500 ctDNA v2 is part of an integrated workflow that spans from sample input to results report (Figure 2). Automated library preparation kits and methods, variant calling tools, and interpretation and reporting software enable a smooth workflow that can be completed in 2.5 days, less than half the time of other liquid biopsy assays (Figure 3).

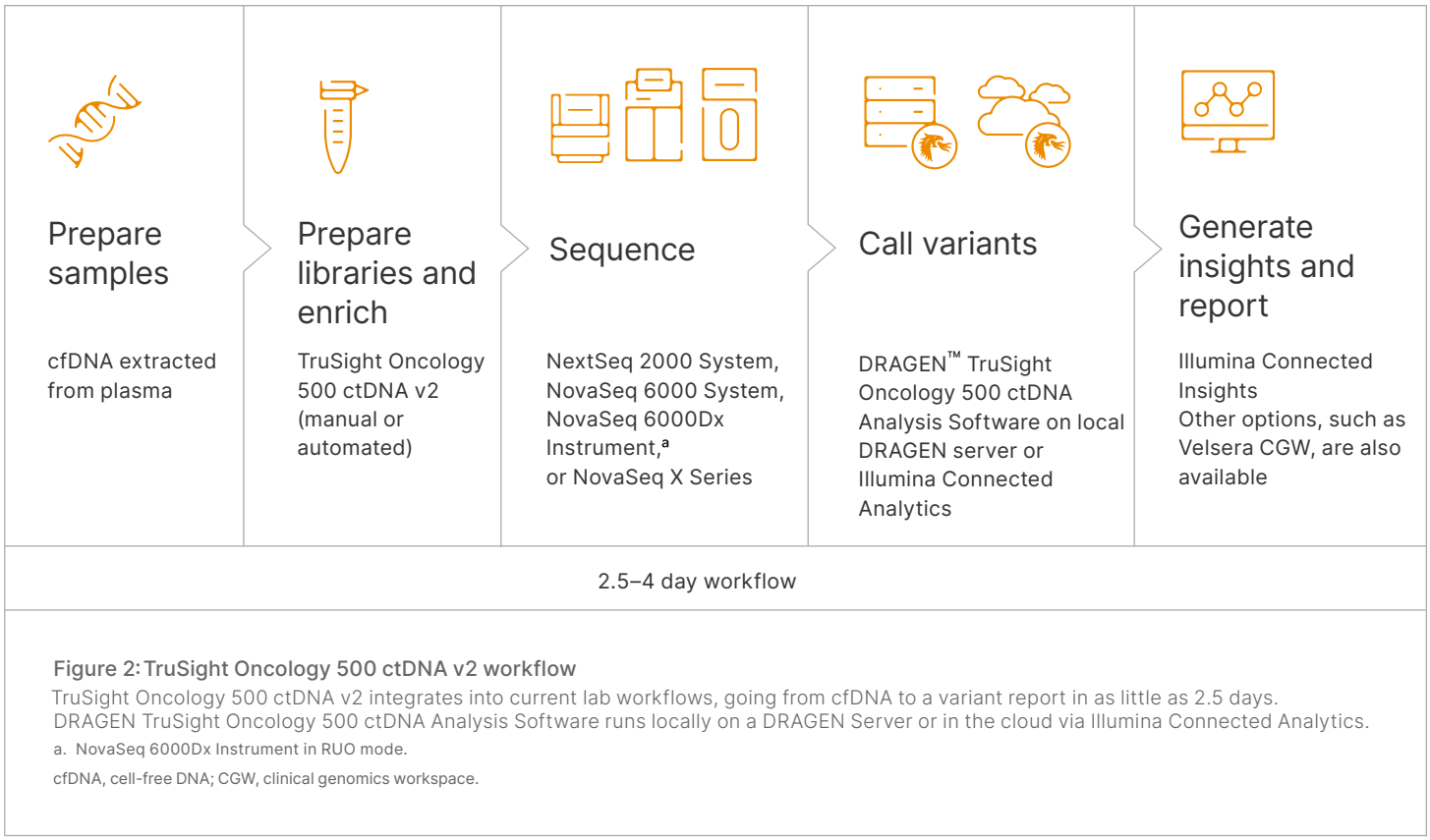

Figure 2: TruSight Oncology 500 ctDNA v2 workflow
TruSight Oncology 500 ctDNA v2 integrates into current lab workflows, going from cfDNA to a variant report in as little as 2.5 days. DRAGEN TruSight Oncology 500 ctDNA Analysis Software runs locally on a DRAGEN Server or in the cloud via Illumina Connected Analytics.

a. NovaSeq 6000Dx Instrument in RUO mode.

cfDNA, cell-free DNA; CGW, clinical genomics workspace.

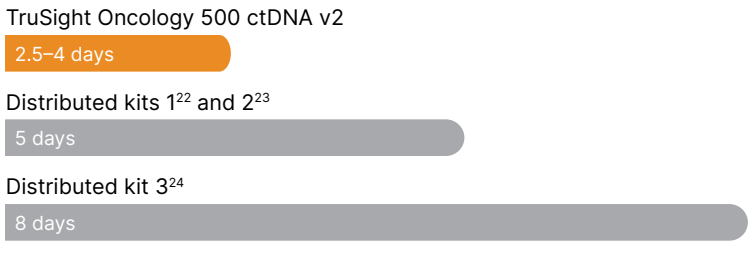

Figure 3: Faster time to results report with TruSight Oncology 500 ctDNA v2
A comparison of the turnaround time from sample to results report for liquid biopsy CGP assays that include the IO biomarkers bMSI and bTMB and other assays. Comparator kits cited are research use only.

bMSI, blood-based microsatellite instability; bTMB, blood-based tumor mutational burden; CGP, comprehensive genomic profiling; IO, immuno-oncology.

Optimized library preparation

Using proven Illumina sequencing by synthesis (SBS) chemistry, TruSight Oncology 500 ctDNA v2 enables CGP from just 20 ng cfDNA, making it an ideal alternative for use when tissue is not readily available or as a complement to tissue studies. Because ctDNA comprises a small fraction of total cfDNA, often less than 5%, powerful methods are required to separate signal from noise. To enable ultra-low frequency variant identification, library preparation uses target enrichment with biotinylated probes and streptavidin-coated magnetic beads to enrich for selected targets from DNA-based libraries (Figure 4). Unique molecular identifiers (UMIs) are also incorporated to reduce errors.[21] Advances in the product chemistry have decreased the number of hybridizations from two to one, allowing for a one-day turnaround time for library preparation and a shorter time to results. Analytical sensitivity is also improved, down to 0.2% variant allele frequency (VAF) for SNVs. This targeted hybridization–capture approach reduces sample dropouts in the presence of both natural allelic variations and sequence artifacts.

Learn about using input amounts as low as 5 ng with TruSight Oncology ctDNA v2.

Learn about UMIs.

Automation-enabled workflow

TruSight Oncology 500 ctDNA v2 supports both manual and automated library preparation to enable scalable workflows. Illumina has partnered with Hamilton and Beckman Coulter Life Sciences, both leaders in liquid handling automation, to develop fully automated workflows for the assay on the Hamilton NGS STAR MOA and the Beckman Coulter Biomek i7 platforms. The automation-compatible library preparation kits include sufficient reagents to prepare 48 libraries while accounting for the dead volume required by automated systems, minimizing reagent waste. These automated workflows deliver the same high-quality results as manual protocols while reducing hands-on time by approximately 40%. In addition, automation helps laboratories lower labor costs and improve operational efficiency.[26]

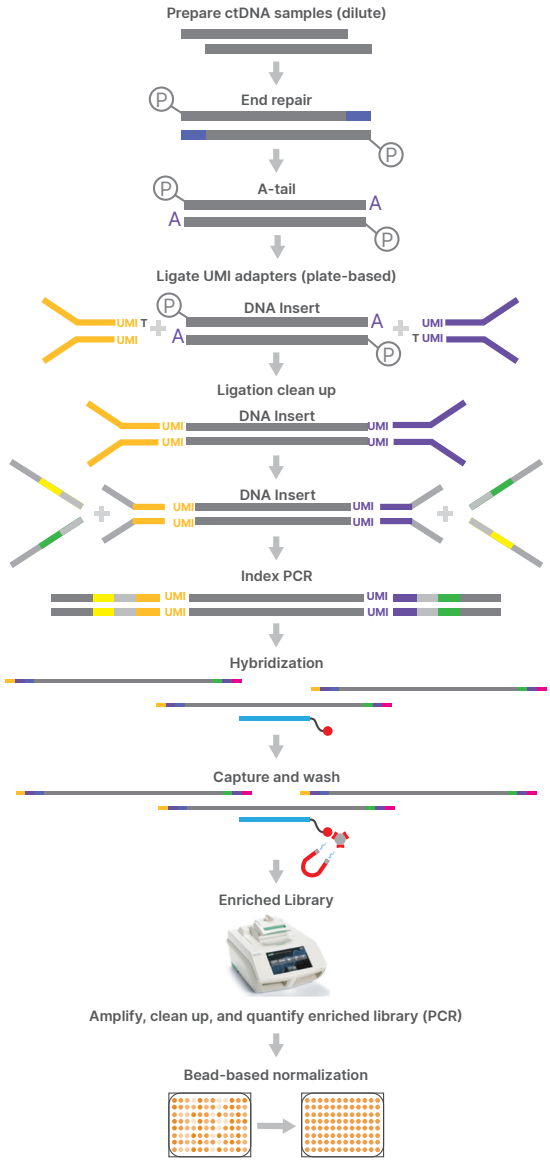

Figure 4: UMI-based hybrid–capture library preparation

Targeted enrichment uses probes designed to provide high binding specificity while still allowing hybridization to target sequences containing variants. Addition of UMIs reduces errors, increasing analytical specificity and yielding higher confidence variant calls.[25]

UMI, unique molecular identifier.

Powerful sequencing

TruSight Oncology 500 ctDNA v2 libraries are sequenced on the NextSeq 2000 System, NovaSeq 6000 System, NovaSeq 6000Dx Instrument (RUO mode), or NovaSeq X Series.

The NextSeq 2000 System and the NovaSeq X Series use XLEAP-SBS™ chemistry, which provides higher output, speed, and cost-effectiveness. When running TruSight Oncology 500 ctDNA v2 on the NovaSeq X Series, users benefit from transformative economics by reducing the cost of sequencing per sample.[27] In addition, the NovaSeq X Series offers a faster workflow, shortening sequencing time by approximately 40% compared to the NovaSeq 6000 System (Table 6).

Implementation of TruSight Oncology 500 ctDNA v2 on the NextSeq 2000 System makes CGP from liquid biopsy samples accessible to laboratories that prefer a benchtop instrument for low- and mid-throughput sequencing. Even with smaller batches, the NextSeq 2000 System enables cost-effective sequencing and supports completion of the entire workflow, from extracted nucleic acids to insights, in three days.

Regardless of the platform used, sequencing occurs at high depth (400M reads per sample at ~35,000×) to enhance sensitivity. As a result, variants can be detected at 0.2% VAF for SNVs, with ≥ 90% analytical sensitivity and ≥ 99% analytical specificity (Table 3).

Accurate, accelerated analysis

Comprehensive, efficient variant calling

DRAGEN TruSight Oncology 500 ctDNA Analysis Software uses accelerated, fully integrated 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.

Table 6: End-to-end turnaround times

Configuration			Run time				
System	Flow cell	No. of samples	Library prep (manual)	Sequencing	DRAGEN secondary analysis[a]	Connected Insights case reporting	Total
NextSeq 2000	P4	4	8.5 hr	44 hr	1 hr 45 min	20 min	~ 3 days
NovaSeq 6000/ NovaSeq 6000Dx[b]	S1	4	8.5 hr	25 hr	1 hr 45 min	20 min	~ 2.5 days
	S2	8	8.5 hr	36 hr	2 hr 15 min	50 min	~ 3 days
	S4	24	8.5 hr	44 hr	3 hr	2 hr 30 min	~ 3.5 days
NovaSeq X	1.5B	4	8.5 hr	22 hr	1 hr 45 min	20 min	~ 2.5 days
	10B	24	8.5 hr	25 hr	3 hr	2 hr 30 min	~ 3 days
	25B	64	10 hr	48 hr	4 hr 10 min	6 hr 40 min	~ 4 days

a. Times listed correspond to analysis time in Illumina Connected Analytics (cloud) and include 0.5 hr queuing time; queue times may vary.
b. NovaSeq 6000Dx Instrument in RUO mode.

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 Software runs locally on an Illumina DRAGEN Server v4 or in the cloud via Illumina Connected Analytics, which offers automated data transfer and analysis launch. This secure, scalable cloud-based solution both speeds up secondary analysis (Table 7) and eliminates the need to acquire and maintain local infrastructure.[28]

Table 7: Analysis time for DRAGEN TruSight Oncology 500 ctDNA software[a]

No. of samples	Illumina Connected Analytics (cloud)[b]	DRAGEN server (local)
4	1 hr 45 min	3 hr
8	2 hr 15 min	7 hr
24	3 hr	19 hr
64	4 hr 10 min	43 hr

a. Times were generated using v2.6.3
b. Times listed include 0.5 hr queuing time; queue times may vary.

Streamlined data interpretation

After variants are identified through secondary analysis, the next step is to derive biologically meaningful insights. TruSight Oncology 500 ctDNA v2 can be used with multiple commercial variant interpretation solutions. Illumina Connected Insights offers the most integrated experience for DRAGEN TruSight Oncology 500 ctDNA data and enables a fully automated analysis workflow that eliminates the need for manual data transfers.

The Connected Insights software incorporates more than 55 knowledge sources, including the Cancer Knowledgebase (CKB) by Genomenon and OncoKB by the Memorial Sloan Kettering Cancer Center, to support variant interpretation. It also allows laboratories to curate and reuse their own variant classifications. Illumina Connected Insights is optimized to work with TruSight Oncology 500 ctDNA v2 data. For example, since bMSI status is determined using the Jensen-Shannon distance (JDS) sum score, Illumina Connected Insights allows users to set bMSI thresholds for TruSight Oncology 500 ctDNA v2 independently from those used for tissue biopsy assays, which often rely on the percentage of unstable sites to determine bMSI status.

Visualization tools are also optimized for TruSight Oncology 500 ctDNA v2 data. In addition to coverage graphs (Figure 5) and a genome view, Illumina Connected Insights includes fusion plots to help users with quality control and interpretation of gene rearrangements. The plots display breakpoints, reading frames, protein domains, supporting reads, and other key metrics (Figure 6).

Variant calling files generated locally or in the cloud with Illumina Connected Analytics can be automatically imported into Illumina Connected Insights. Sequencing system integration and autolaunch features automate the entire analysis workflow, eliminating manual data transfers and generating a final, customizable results report.

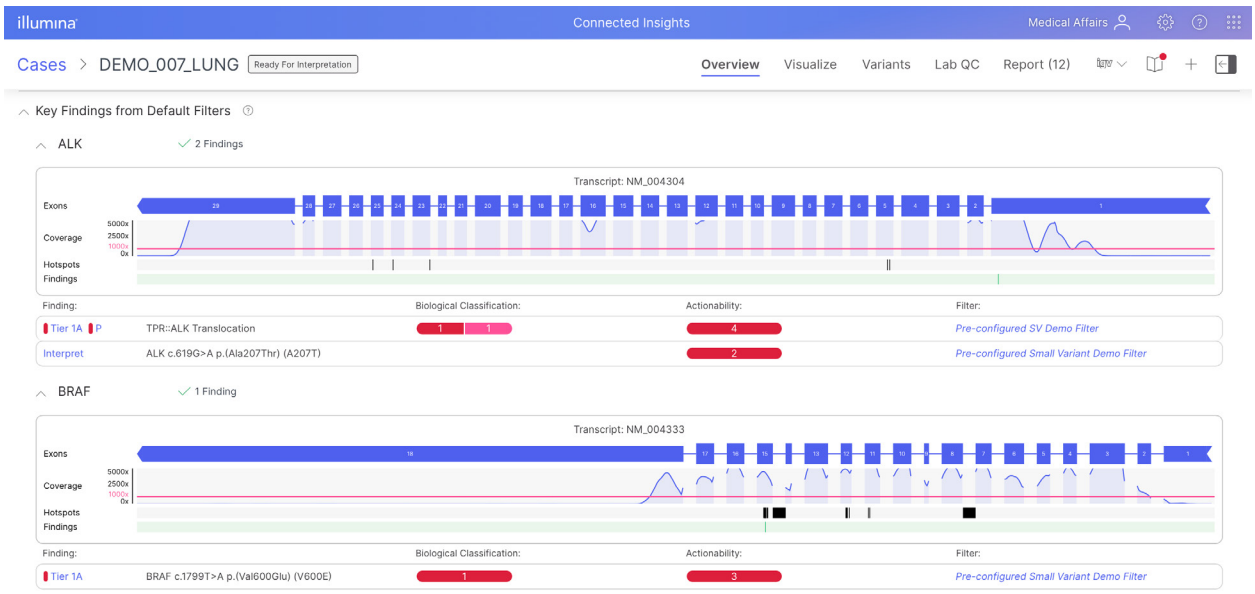

Figure 5: Assessing gene and exon coverage with Illumina Connected Insights
Coverage across targeted genes and exons is displayed to reveal gaps that may impact variant detection and interpretation.

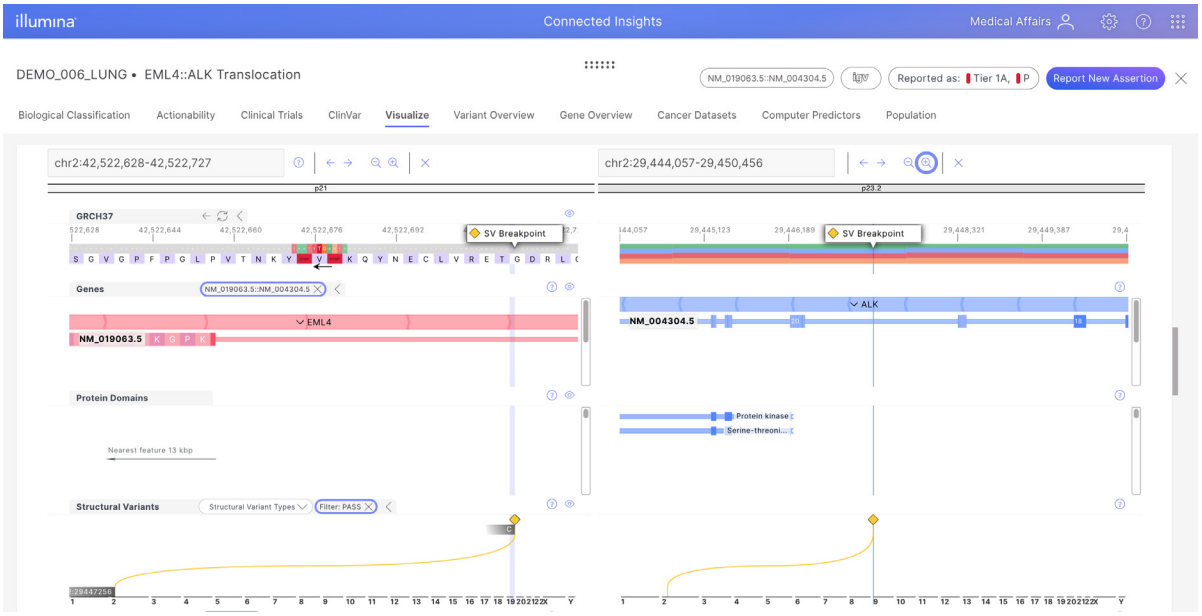

Figure 6: Gene fusion visualization generated by Illumina Connected Insights
Fusion partners, breakpoints, and supporting read evidence are displayed to assist with quality control and interpretation.

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, bTMB, and bMSI. Performance results were verified on the NextSeq 2000 System, NovaSeq 6000 System, and NovaSeq X Series.

SNVs and indels

One advantage of target enrichment chemistry is the use of probes designed to be large enough to ensure high binding specificity, while still allowing hybridization to targets containing small mutations. Because SNVs are associated with cancer susceptibility across multiple tumor types, CGP methods must reliably detect these variants at low levels. TruSight Oncology 500 ctDNA v2 detects SNVs and indels present at levels as low as 0.2% or 0.5% VAF, respectively, with high reproducibility (see Figure 7 and Figure 8 for near LOD performance).

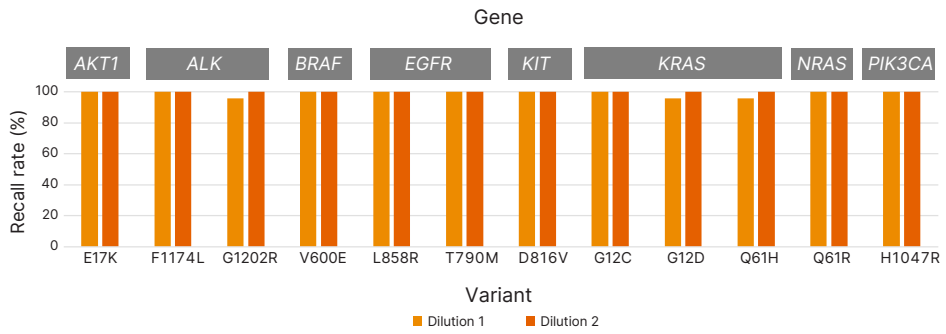

Figure 7: High analytical performance for key SNVs near LOD (0.2% VAF)

Synthetic control samples containing known SNVs were diluted to VAF levels ranging from 0.20% to 0.50% and analyzed with TruSight Oncology 500 ctDNA v2. Dilution 1 had a mean VAF of 0.24% (95% C.I. 0.23–0.25) and Dilution 2 had a mean VAF of 0.49% (95% C.I. 0.48–0.52). SNVs were reliably detected at low VAF levels. Results shown were generated on the NovaSeq 6000 System, with equivalent performance observed on the NextSeq 2000 System and NovaSeq X Series.

LOD, limit of detection; SNV, single nucleotide variant; VAF, variant allele frequency.

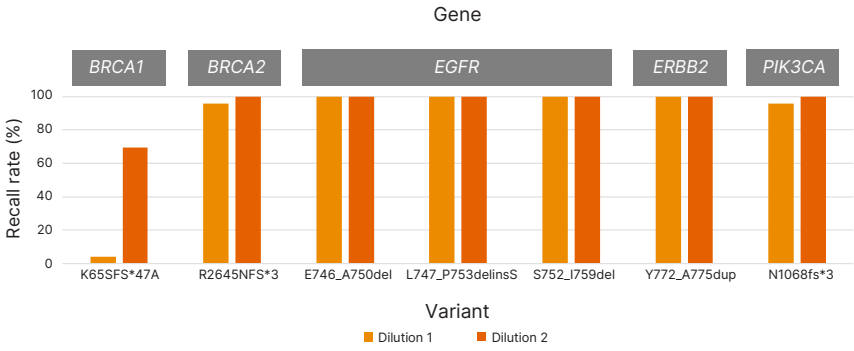

Figure 8: High analytical performance for indels near LOD (0.5% VAF)

Synthetic control samples containing known insertions and deletions (indels) were diluted targeting VAF levels ranging from 0.20% to 0.50% and analyzed with TruSight Oncology 500 ctDNA v2. Dilution 1 had a mean VAF of 0.29% (95% C.I. 0.26–0.32) and Dilution 2 had a mean VAF of 0.58% (95% C.I. 0.54–0.63). Indels were reliably detected at low VAF levels. Detection of the *BRCA1* variant was lower due to the location of the variant within a highly homopolymeric region, which contributes to elevated background noise. Results shown were generated on the NovaSeq 6000 System, with equivalent performance observed on the NextSeq 2000 System and NovaSeq X Series.

LOD, limit of detection; VAF, variant allele frequency.

CNVs

Copy number changes in genes and tumor types have been linked to tumorigenesis.[27] TruSight Oncology 500 ctDNA v2 includes analysis of 59 CNV-associated genes and can call CNVs with a limit of detection at ≥ 1.3 fold for amplifications and ≤ 0.6 for deletions (Table 8).

Table 8: 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 containing known gene amplifications, generated from synthetic controls, and known deletions, derived from well-characterized cell lines, were evaluated using TruSight Oncology 500 ctDNA v2 across three VAF levels. The assay demonstrated a LOD of ≥ 1.3-fold change for amplifications and ≤ 0.6-fold change for deletions, with strong concordance between expected and observed results. Sequencing data were obtained on the NovaSeq 6000 System; similar performance was observed on the NextSeq 2000 System and the NovaSeq X Series.

CNV, copy number variant; LOD, limit of detection; N/A, not applicable.

Gene rearrangements

Gene rearrangements are key genomic drivers of cancer, making sensitive detection critical for studies investigating disease mechanisms. TruSight Oncology 500 ctDNA v2 enables detection and characterization of gene rearrangements independent of the fusion partner, even at low concentrations (Table 9).

Table 9: 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%

TruSight Oncology 500 ctDNA v2 reliably detected three known DNA fusions diluted to VAF levels as low as 0.2%, with a demonstrated LOD of 0.5% for gene rearrangements. Sequencing data were obtained on the NovaSeq 6000 System; similar performance was observed on the NextSeq 2000 System and the NovaSeq X Series.

LOD, limit of detection; VAF, variant allele frequency.

IO gene signatures: bMSI and bTMB

bMSI and bTMB detection requires analysis across numerous genomic loci. TruSight Oncology 500 ctDNA v2 enables NGS-based assessment of more than 2300 homopolymer sites (6–7 bp in length), reducing error rates and minimizing false positives commonly associated with homopolymer sequencing.[27] Featuring sensitive library preparation chemistry and advanced bioinformatics, TruSight Oncology 500 ctDNA v2 achieves bMSI detection down to 0.3% tumor fraction (Figure 9).

Accurately and reproducibly determining bTMB at low mutation levels can be challenging with smaller panels.[7] TruSight Oncology 500 ctDNA v2 overcomes this by combining broad genomic coverage through a 1.94 Mb panel combined with advanced bioinformatics. The proprietary DRAGEN TruSight Oncology 500 ctDNA pipeline applies optimized filtering to exclude germline and clonal hematopoiesis–associated variants, delivering highly concordant tumor-only and tumor–normal workflows (R^2 = 0.992) (Figure 10).[29]

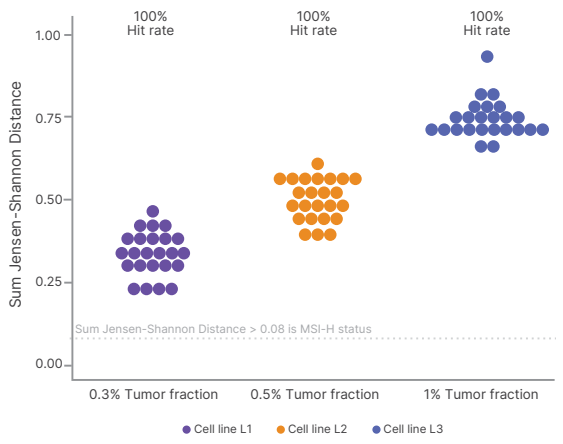

Figure 9: High bMSI analytical sensitivity achieved with TruSight Oncology 500 ctDNA v2

Tumor fractions were generated by titrating nucleosomal-prepared cell lines with known MSI-H status into a wild type cell background. High bMSI analytical sensitivity was achieved using the proprietary DRAGEN TruSight Oncology 500 ctDNA Analysis Software. More than 2300 homopolymer sites were assessed. Sequencing data were obtained on the NovaSeq 6000 System; similar performance was observed on the NextSeq 2000 System and NovaSeq X Series.

CGP, comprehensive genomic profiling; bMSI, blood-based microsatellite instability, MSI-H, microsatellite instability-high.

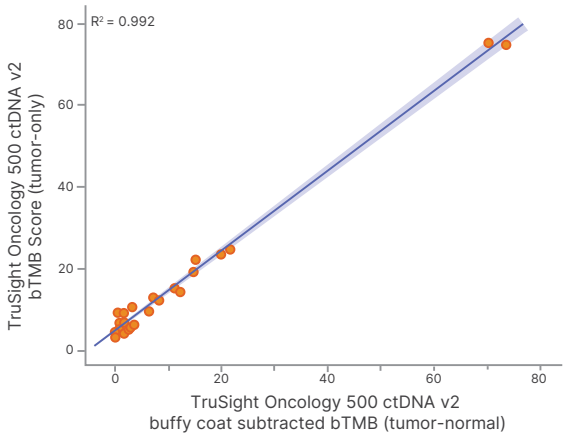

Figure 10: High bTMB data correlation between tumor-only and tumor-normal analysis workflows

Tumor-only bTMB scores generated with TruSight Oncology 500 ctDNA v2 show high concordance to bTMB scores produced from a paired tumor–normal workflow using plasma and buffy coat cfDNA. Sequencing data were obtained on the NovaSeq 6000 System. bTMB scores were highly concordant with those obtained using the NextSeq 2000 System and NovaSeq X Series.

bTMB, blood-based tumor mutational burden.

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 in advance of any significant changes to TruSight Oncology 500 ctDNA v2
- Certificate of Analysis—TruSight Oncology 500 ctDNA v2 includes a certificate of analysis (CoA) that confirms the product meets predefined release specifications and quality standards
- Extended shelf life—TruSight Oncology 500 ctDNA v2 reagents have a minimum guaranteed shelf life of six months, reducing the risk of expiration and supporting flexible testing schedules
- Single-lot shipments— TruSight Oncology 500 ctDNA v2 manual kits are available as single-lot shipments to reduce lot qualification and incoming QC burden; automation kit requests may be accommodated on a case-by-case basis via customer care

Integrated solution enabling CGP from liquid biopsy

TruSight Oncology 500 ctDNA v2 is an NGS-based, multiplex research assay that enables simultaneous analysis of hundreds of cancer-related biomarkers from plasma samples, in alignment with current guidelines and evidence from clinical trials. The comprehensive assay detects multiple variant types in blood across 523 genes implicated in diverse tumor types. It also assesses IO and emerging biomarkers, including bTMB, bMSI, *NTRK*, and *ROS1*, all from a single sample, eliminating the need for iterative testing.

The updated assay chemistry and expanded sequencing system compatibility have decreased overall turnaround time to 2.5–4 days, reduced the input requirement to 20 ng cfDNA, and lowered the limit of detection to 0.2% VAF (for SNVs). The automation-enabled workflow further reduces hands-on time and minimizes the burden on laboratory personnel, improving overall efficiency. By combining broad genomic content, proven sequencing technology, and improved software, TruSight Oncology 500 ctDNA v2 simplifies CGP-based research while delivering superior performance for liquid biopsy studies.

Learn more →

TruSight Oncology 500 ctDNA v2

NovaSeq 6000 System

NovaSeq 6000Dx Instrument

NextSeq 2000 System

NovaSeq X Series

DRAGEN secondary analysis

Illumina Connected Analytics

Illumina Connected Insights

Illumina Automated Solutions

Appendix - TruSight Oncology 500 ctDNA v2 gene list[a]

ABL1	**BCR**	**CHEK1**	**EPHA7**	**FGF8**	**GSK3B**	**IDH2**	**MAP3K1**	**NF2**	**PIK3CA**	**RAD51D**	**SMAD4**	**TGFBR2**
ABL2	**BIRC3**	**CHEK2**	**EPHB1**	FGF9	**H3F3A**	**IFNGR1**	**MAP3K13**	**NFE2L2**	**PIK3CB**	**RAD52**	**SMARCA4**	**TMEM127**
ACVR1	**BLM**	CIC	**ERBB2**	FGF10	**H3F3B**	**INHBA**	**MAP3K14**	**NFKBIA**	**PIK3CD**	**RAD54L**	**SMARCB1**	TMPRSS2
ACVR1B	**BMPR1A**	**CREBBP**	**ERBB3**	**FGF14**	**H3F3C**	**INPP4A**	**MAP3K4**	**NKX2-1**	**PIK3CG**	**RAF1**	**SMARCD1**	**TNFAIP3**
AKT1	BRAF	**CRKL**	**ERBB4**	FGF19	**HGF**	**INPP4B**	**MAPK1**	**NKX3-1**	**PIK3R1**	**RANBP2**	**SMC1A**	**TNFRSF14**
AKT2	**BRCA1**	**CRLF2**	**ERCC1**	FGF23	**HIST1H1C**	**INSR**	**MAPK3**	**NOTCH1**	**PIK3R2**	**RARA**	**SMC3**	**TOP1**
AKT3	**BRCA2**	**CSF1R**	**ERCC2**	**FGFR1**	**HIST1H2BD**	**IRF2**	**MAX**	NOTCH2	**PIK3R3**	**RASA1**	SMO	**TOP2A**
ALK	**BRD4**	**CSF3R**	**ERCC3**	**FGFR2**	**HIST1H3A**	**IRF4**	**MCL1**	**NOTCH3**	**PIM1**	**RB1**	**SNCAIP**	**TP53**
ALOX12B	**BRIP1**	**CSNK1A1**	**ERCC4**	**FGFR3**	**HIST1H3B**	**IRS1**	**MDC1**	**NOTCH4**	**PLCG2**	**RBM10**	**SOCS1**	**TP63**
ANKRD11	**BTG1**	**CTCF**	**ERCC5**	**FGFR4**	**HIST1H3C**	**IRS2**	**MDM2**	**NPM1**	**PLK2**	**RECQL4**	**SOX10**	**TRAF2**
ANKRD26	**BTK**	**CTLA4**	**ERG**	**FH**	**HIST1H3D**	**JAK1**	MDM4	NRAS	**PMAIP1**	**REL**	**SOX17**	**TRAF7**
APC	**C11orf30**	**CTNNA1**	**ERRFI1**	**FLCN**	**HIST1H3E**	**JAK2**	**MED12**	**NRG1**	**PMS1**	**RET**	**SOX2**	**TSC1**
AR	**CALR**	**CTNNB1**	**ESR1**	**FLI1**	**HIST1H3F**	**JAK3**	**MEF2B**	**NSD1**	PMS2	**RFWD2**	**SOX9**	**TSC2**
ARAF	**CARD11**	**CUL3**	**ETS1**	**FLT1**	**HIST1H3G**	**JUN**	**MEN1**	NTRK1	**PNRC1**	**RHEB**	**SPEN**	**TSHR**
ARFRP1	**CASP8**	**CUX1**	ETV1	**FLT3**	**HIST1H3H**	**KAT6A**	**MET**	**NTRK2**	**POLD1**	**RHOA**	**SPOP**	**U2AF1**
ARID1A	**CBFB**	**CXCR4**	**ETV4**	**FLT4**	**HIST1H3I**	**KDM5A**	**MGA**	**NTRK3**	**POLE**	**RICTOR**	**SPTA1**	**VEGFA**
ARID1B	**CBL**	**CYLD**	**ETV5**	**FOXA1**	**HIST1H3J**	**KDM5C**	**MITF**	**NUP93**	PPARG	**RIT1**	**SRC**	**VHL**
ARID2	CCND1	**DAXX**	ETV6	**FOXL2**	**HIST2H3A**	**KDM6A**	**MLH1**	NUTM1	**PPM1D**	**RNF43**	**SRSF2**	**VTCN1**
ARID5B	**CCND2**	**DCUN1D1**	EWSR1	**FOXO1**	**HIST2H3C**	**KDR**	**MLL**	PAK1	**PPP2R1A**	**ROS1**	**STAG1**	**WISP3**
ASXL1	CCND3	**DDR2**	**EZH2**	**FOXP1**	**HIST2H3D**	**KEAP1**	MLLT3	**PAK3**	**PPP2R2A**	**RPS6KA4**	**STAG2**	**WT1**
ASXL2	CCNE1	**DDX41**	**FAM123B**	**FRS2**	**HIST3H3**	**KEL**	**MPL**	**PAK7**	**PPP6C**	RPS6KB1	**STAT3**	**XIAP**
ATM	**CD274**	**DHX15**	**FAM175A**	**FUBP1**	HLA-A	**KIF5B**	**MRE11A**	**PALB2**	**PRDM1**	**RPS6KB2**	**STAT4**	**XPO1**
ATR	**CD276**	**DICER1**	**FAM46C**	**FYN**	HLA-B	**KIT**	**MSH2**	**PARK2**	**PREX2**	**RPTOR**	**STAT5A**	**XRCC2**
ATRX	CD74	**DIS3**	**FANCA**	**GABRA6**	HLA-C	**KLF4**	**MSH3**	**PARP1**	**PRKAR1A**	**RUNX1**	**STAT5B**	**YAP1**
AURKA	**CD79A**	**DNAJB1**	**FANCC**	**GATA1**	**HNF1A**	**KLHL6**	**MSH6**	PAX3	**PRKCI**	**RUNX1T1**	**STK11**	**YES1**
AURKB	**CD79B**	**DNMT1**	**FANCD2**	**GATA2**	**HNRNPK**	KMT2B	MST1	**PAX5**	**PRKDC**	**RYBP**	**STK40**	**ZBTB2**
AXIN1	**CDC73**	**DNMT3A**	**FANCE**	**GATA3**	**HOXB13**	KMT2C	**MST1R**	**PAX7**	**PRSS8**	**SDHA**	**SUFU**	**ZBTB7A**
AXIN2	**CDH1**	**DNMT3B**	**FANCF**	**GATA4**	**IGF1**	KMT2D	**MTOR**	**PAX8**	**PTCH1**	**SDHAF2**	**SUZ12**	**ZFHX3**
AXL	**CDK12**	**DOT1L**	**FANCG**	**GATA6**	**IGF1R**	KRAS	**MUTYH**	**PBRM1**	**PTEN**	**SDHB**	**SYK**	**ZNF217**
B2M	**CDK4**	E2F3	**FANCI**	**GEN1**	**IGF2**	LAMP1	**MYB**	**PDCD1**	**PTPN11**	**SDHC**	**TAF1**	**ZNF703**
BAP1	**CDK6**	**EED**	**FANCL**	**GID4**	**IKBKE**	**LATS1**	**MYC**	**PDCD1LG2**	**PTPRD**	**SDHD**	**TBX3**	**ZRSR2**
BARD1	**CDK8**	**EGFL7**	**FAS**	**GLI1**	**IKZF1**	**LATS2**	**MYCL1**	**PDGFRA**	**PTPRS**	**SETBP1**	**TCEB1**	
BBC3	**CDKN1A**	EGFR	**FAT1**	**GNA11**	**IL10**	**LMO1**	MYCN	**PDGFRB**	**PTPRT**	**SETD2**	**TCF3**	
BCL10	**CDKN1B**	**EIF1AX**	**FBXW7**	**GNA13**	**IL7R**	**LRP1B**	**MYD88**	**PDK1**	**QKI**	**SF3B1**	**TCF7L2**	
BCL2	**CDKN2A**	**EIF4A2**	**FGF1**	GNAQ	**INHA**	**LYN**	**MYOD1**	**PDPK1**	**RAB35**	**SH2B3**	TERC	
BCL2L1	**CDKN2B**	**EIF4E**	**FGF2**	**GNAS**	**HRAS**	**LZTR1**	**NAB2**	**PGR**	**RAC1**	**SH2D1A**	**TERT**[b]	
BCL2L11	**CDKN2C**	**EML4**	**FGF3**	**GPR124**	**HSD3B1**	**MAGI2**	**NBN**	**PHF6**	**RAD21**	**SHQ1**	**TET1**	
BCL2L2	CEBPA	**EP300**	**FGF4**	**GPS2**	**HSP90AA1**	**MALT1**	**NCOA3**	PHOX2B	**RAD50**	**SLIT2**	**TET2**	
BCL6	**CENPA**	**EPCAM**	FGF5	**GREM1**	**ICOSLG**	**MAP2K1**	**NCOR1**	**PIK3C2B**	**RAD51**	**SLX4**	TFE3	
BCOR	**CHD2**	**EPHA3**	FGF6	**GRIN2A**	**ID3**	**MAP2K2**	**NEGR1**	**PIK3C2G**	**RAD51B**	**SMAD2**	**TFRC**	
BCORL1	**CHD4**	**EPHA5**	FGF7	**GRM3**	**IDH1**	**MAP2K4**	NF1	**PIK3C3**	**RAD51C**	**SMAD3**	**TGFBR1**	

a. TruSight Oncology 500 ctDNA v2 detects small variants for all genes listed.
b. Only the *TERT* promoter region is covered for variant calling.

Light orange boxes indicate genes that include copy number variants.
Yellow boxes indicate genes that include DNA fusions.
Dark orange boxes indicate genes that include both copy number variants and DNA fusions.
Probes target at least 97% of the coding sequence for all genes in bold.

Ordering information—Library preparation kits (manual)

Product	Catalog no.
TruSight Oncology 500 ctDNA v2 (24 samples)	20105899
TruSight Oncology 500 ctDNA v2 (24 samples) plus Illumina Connected Insights Software	20105911
TruSight Oncology 500 ctDNA v2 (24 samples) plus Velsera Interpretation Report	20105905
TruSight Oncology 500 ctDNA v2 for use with NextSeq 2000 P4 (24 samples)	20151788
TruSight Oncology 500 ctDNA v2 plus Illumina Connected Insights Software, for use with NextSeq 2000 P4 (24 samples)	20151792
TruSight Oncology 500 ctDNA v2 (24 samples) plus Velsera Interpretation Report, for use with for use with NextSeq 2000 P4 (24 samples)	20151790
TruSight Oncology 500 ctDNA v2 for use with NovaSeq 6000 S2 (24 samples)	20105901
TruSight Oncology 500 ctDNA v2 plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S2 (24 samples)	20105913
TruSight Oncology 500 ctDNA v2 plus Velsera Interpretation Report, for use with NovaSeq 6000 S2 (24 samples)	20105907
TruSight Oncology 500 ctDNA v2 for use with NovaSeq 6000 S4 (24 samples)	20105902
TruSight Oncology 500 ctDNA v2 plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S2 (24 samples)	20105913
TruSight Oncology 500 ctDNA v2 plus Velsera Interpretation Report, for use with NovaSeq 6000 S4 (24 samples)	20105908
TruSight Oncology 500 ctDNA v2 plus Connected Insights Interpretation Report, for use with NovaSeq 6000 S4 (24 samples)	20105914

Ordering information—Library preparation kits (automated)

Product	Catalog no.
TruSight Oncology 500 ctDNA v2 for Automation (48 samples)	20105900
TruSight Oncology 500 ctDNA v2 for Automation (48 samples) plus Illumina Connected Insights Software	20105912
TruSight Oncology 500 ctDNA v2 for Automation (48 samples) plus Velsera Interpretation Report	20105906
TruSight Oncology 500 ctDNA v2 Automation Kit, for use with NovaSeq 6000 S2 (48 samples)	20105903
TruSight Oncology 500 ctDNA v2 Automation Kit plus Connected Insights Software, for use with NovaSeq 6000 S2 (48 samples)	20105915
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, for use with NovaSeq 6000 S4 (48 samples)	20105904
TruSight Oncology 500 ctDNA v2 Automation Kit plus Illumina Connected Insights Software, for use with NovaSeq 6000 S4 (48 samples)	20105916
TruSight Oncology 500 ctDNA v2 Automation Kit plus Velsera Interpretation Report, for use with NovaSeq 6000 S4 (48 samples)	20105910

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
Illumina UMI DNA/RNA UD v3 indexes Set A, Ligation (96 indexes, 96 Samples)	20126235
Illumina UMI DNA/RNA UD v3 indexes Set B, Ligation (96 indexes, 96 Samples)	20126237
Illumina UMI DNA/RNA UD indexes v3, Set A, Auto (96 indexes, 96 Samples)	20141200
Illumina UMI DNA/RNA UD indexes v3, Set B, Auto (96 indexes, 96 Samples)	20141201

Ordering information—Sequencing reagents

Product	Catalog no.
NextSeq 2000 P4 XLEAP-SBS Reagent Kit (300 Cycles)	20100992
NovaSeq 6000 S1 Reagent Kit v1.5 (300 cycles)	20028317
NovaSeq 6000 S2 Reagent Kit v1.5 (300 cycles)	20028314
NovaSeq 6000 S4 Reagent Kit v1.5 (300 cycles)	20028312
NovaSeq X Series 1.5B Reagent Kit (300 cycles)	20104705
NovaSeq X Series 10B Reagent Kit (300 cycles)	20085594
NovaSeq X Series 25B Reagent Kit (300 cycles)	20104706

Ordering information—Analysis

Product	Catalog no.
Local secondary analysis	
Illumina DRAGEN Server v4	20051343
Illumina DRAGEN Server Installation	20031995
Illumina DRAGEN Server v4 Support Plan	20085832
Field Delivered Applications Training	15032919
Cloud-based secondary analysis	
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	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 – 1000 iCredits	20042039
Illumina Analytics – 5000 iCredits	20042040
Illumina Analytics – 50,000 iCredits	20042041
Illumina Analytics – 100,000 iCredits	20042042

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