

# TruSight™ Oncology 500 v2

Enabling faster, streamlined CGP  
with enhanced sensitivity and  
HRD analysis



Analyze key variant classes,  
immuno-oncology signatures,  
and HRD in a single assay



Go from sample to results in  
3–4 days with manual or  
automated options



Obtain more sensitive profiling  
from FFPE samples

## Introduction

Comprehensive genomic profiling (CGP) has emerged as a critical tool in research laboratories, offering a broad assessment of the genomic landscape across a broad range of diseases, particularly cancer.<sup>1</sup> By simultaneously evaluating hundreds of genes for mutations, copy number variations, gene fusions, and other genomic alterations, CGP can provide a holistic view of the molecular drivers of disease.

The original Illumina TruSight Oncology 500, released in 2018, is a next-generation sequencing (NGS) assay for detecting pan-cancer biomarkers, including DNA and RNA variant types, microsatellite instability (MSI), and tumor mutational burden (TMB). TruSight Oncology 500 has been successfully adopted by research laboratories worldwide, enabling reliable and large-scale genomic profiling.<sup>2-5</sup>

Building on this success, Illumina offers TruSight Oncology 500 v2. This research assay improves upon the original TruSight Oncology 500 by incorporating a single-hybridization enrichment workflow, providing reduced hands-on time and an overall faster time to results (Table 1 and Table 2). TruSight Oncology 500 v2 includes a fully integrated homologous recombination deficiency (HRD) panel. HRD results from a cell's inability to repair double-stranded DNA breaks, leading to genomic instability that drives cancer development.<sup>6</sup>

## Comprehensive content design

The increasing number of key genetic alterations in both DNA and RNA underscores the importance of comprehensive assays that analyze both analytes in a single workflow.<sup>7</sup> The TruSight Oncology 500 v2 assay includes a broad range of variant types and biomarkers, with HRD assessment now fully integrated (Figure 1).

Illumina partnered with recognized authorities in the oncology community to design the TruSight Oncology 500 v2 content. The resulting panel provides comprehensive tumor profiling of biomarkers, with coverage of 523 genes that are frequently mutated in cancer. Biomarkers called from DNA include single nucleotide variants (SNVs), insertions/deletions (indels), copy number variants (CNVs), and gene signatures, including TMB, MSI, and HRD.<sup>8-10</sup> Biomarkers called from RNA include known and novel gene fusions, and splice variants in 55 genes (Appendix).

The panel content comprises genes listed in current guidelines with significant coverage of key guidelines for multiple tumor types (Figure 2) and genes involved in over 1000 clinical trials.

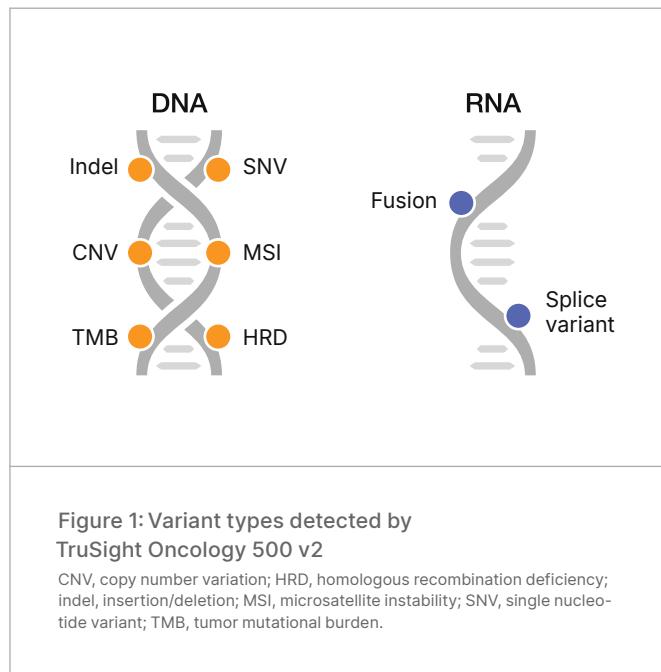


Figure 1: Variant types detected by TruSight Oncology 500 v2

CNV, copy number variation; HRD, homologous recombination deficiency; indel, insertion/deletion; MSI, microsatellite instability; SNV, single nucleotide variant; TMB, tumor mutational burden.

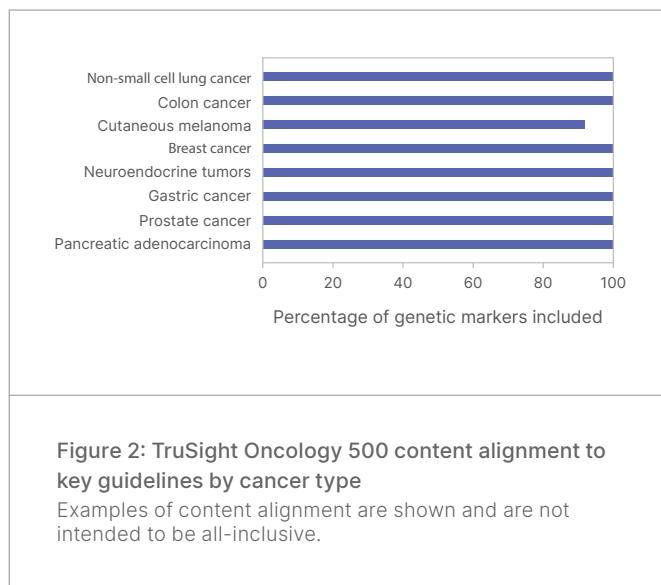


Table 1: TruSight Oncology 500 v2 specifications

Parameter	TruSight Oncology 500 v2			
System	NextSeq™ 550 or 550Dx <sup>a</sup> Systems	NextSeq 1000 or 2000 Systems	NovaSeq™ 6000 or 6000Dx <sup>a</sup> Systems	NovaSeq X Series
Sample throughput	8 samples per flow cell	8–36 samples per flow cell	16–192 samples per flow cell	32–480 samples per flow cell
Sequencing run time (flow cell)	24 hr	19 hr (P2) 31 hr (P3) 34 hr (P4)	19 hr (SP and S1) 25 hr (S2) 36 hr (S4)	20 hr (1.5B) 22 hr (10B) 38 hr (25B)
DNA input requirement	30 ng recommended (as low as 10 ng)			
RNA input requirement	40 ng recommended (as low as 20 ng)			
Panel size	1.94 Mb DNA, 358 kb RNA, ~25K SNPs HRD			
FFPE input requirement	Minimum recommendation of 2 mm <sup>3</sup> from FFPE tissue samples			
Sequencing run length	2 × 101 cycles			
Software version	DRAGEN™ TruSight Oncology 500 v2.6.2+, Illumina Connected Insights v5.1.1+			
Sensitivity	At least 95% for all variant types at limit of detection			
Hands-on time <sup>b</sup>	<ul style="list-style-type: none"> <li>Manual: ~5–7 hr</li> <li>Automation: 3.25 hr</li> </ul>			
Walk-away time <sup>b</sup>	<ul style="list-style-type: none"> <li>Manual: 12–13 hr</li> <li>Automation: <ul style="list-style-type: none"> <li>Hamilton STAR MOA: 14–16 hr</li> <li>Beckman i7: 15–17.5 hr</li> </ul> </li> </ul>			
Total assay time	3–4 days from nucleic acid to results report			
Total turnaround time	~3–4 days from library prep to insights			
Limit of detection	<ul style="list-style-type: none"> <li>Small variants: 5% VAF</li> <li>Fusions: 17 supporting reads</li> <li>Splice variants: 21 supporting reads</li> <li>CNVs: <ul style="list-style-type: none"> <li>Gene amplifications: 1.8-fold change<sup>c</sup></li> <li>Gene deletions: 0.5-fold change</li> <li>BRCA large rearrangements (≥ 3 exons): 25% VAF</li> <li>BRCA large rearrangements (&lt; 3 exons): 41% VAF</li> </ul> </li> <li>HRD GIS: 23% tumor content</li> <li>MSI-H: 20% tumor content</li> </ul>			
Specificity <sup>d</sup>	<ul style="list-style-type: none"> <li>Small variants: 99.9998%</li> <li>Fusions: 98%</li> <li>Splice variants: 99%</li> <li>CNVs: <ul style="list-style-type: none"> <li>Gene amplifications: 99.9%</li> <li>Gene deletions: 99.98%</li> <li>BRCA large rearrangements: 99%</li> </ul> </li> <li>HRD GIS: 100%</li> </ul>			

a. In research mode.

b. Values were determined from timed runs for batch sizes ranging from 8 DNA and 8 RNA samples to 48 DNA and 48 RNA samples.

c. Based on analysis of genes with probe coverage optimized for CNV calling.

d. Internal limit of blank study results.

CNV, copy number variation; FFPE, formalin-fixed, paraffin-embedded; GIS, genomic instability score; HRD, homologous recombination deficiency; MSI-H, microsatellite instability-high; VAF, variant allele frequency.

Table 2: Advances with TruSight Oncology 500 v2

Benefit	TruSight Oncology 500	TruSight Oncology 500 v2
Assay turnaround time	4–5 days nucleic acid to variant report	3–4 days nucleic acid to variant report
Input	40 ng DNA 40–80 ng RNA	30 ng DNA recommended (as low as 10 ng) 40 ng RNA recommended (as low as 20 ng)
Panel	523 DNA genes, 55 RNA genes	523 DNA genes, 55 RNA genes, and HRD panel
HRD feature	HRD panel available as add-on kit sold separately	HRD probe set is included for optional testing GIS can be reported for every sample
Indexes	16–192 UDIs	384 Illumina UDIs
Automation features	Fixed batch sizes, many tubes per kit  Illumina supported for Hamilton STAR MOA (first tier) and Biomek i7 Beckman Coulter (first tier)	Flexible batching (4–96 samples) Optional overnight processing 1 tube for most reagents Support for uneven DNA, RNA, and HRD libraries 6 kit uses supported Fast reagent plating  Illumina supported for Hamilton STAR MOA (first tier) and Biomek i7 Beckman Coulter (first tier)
Kit features	Many tubes and boxes per kit	Reduced packaging (~50% reduction) One tube per reagent (~70% reduction) Color-coded tubes and name on tube cap

GIS, genomic instability score; HRD, homologous recombination deficiency; UDI, unique dual index.

## Fast, integrated workflow

By consolidating testing into a single assay, TruSight Oncology 500 v2 helps preserve limited samples, such as formalin-fixed, paraffin-embedded (FFPE) tissue, and reduces the need for iterative testing.

TruSight Oncology 500 v2 is part of a fully integrated CGP workflow that spans from sample input to final report ([Figure 3](#)). Using automated library preparation kits and methods, variant calling tools, and interpretation and reporting software enables a smooth workflow that can be completed in as few as 3.5 days ([Table 3](#)).

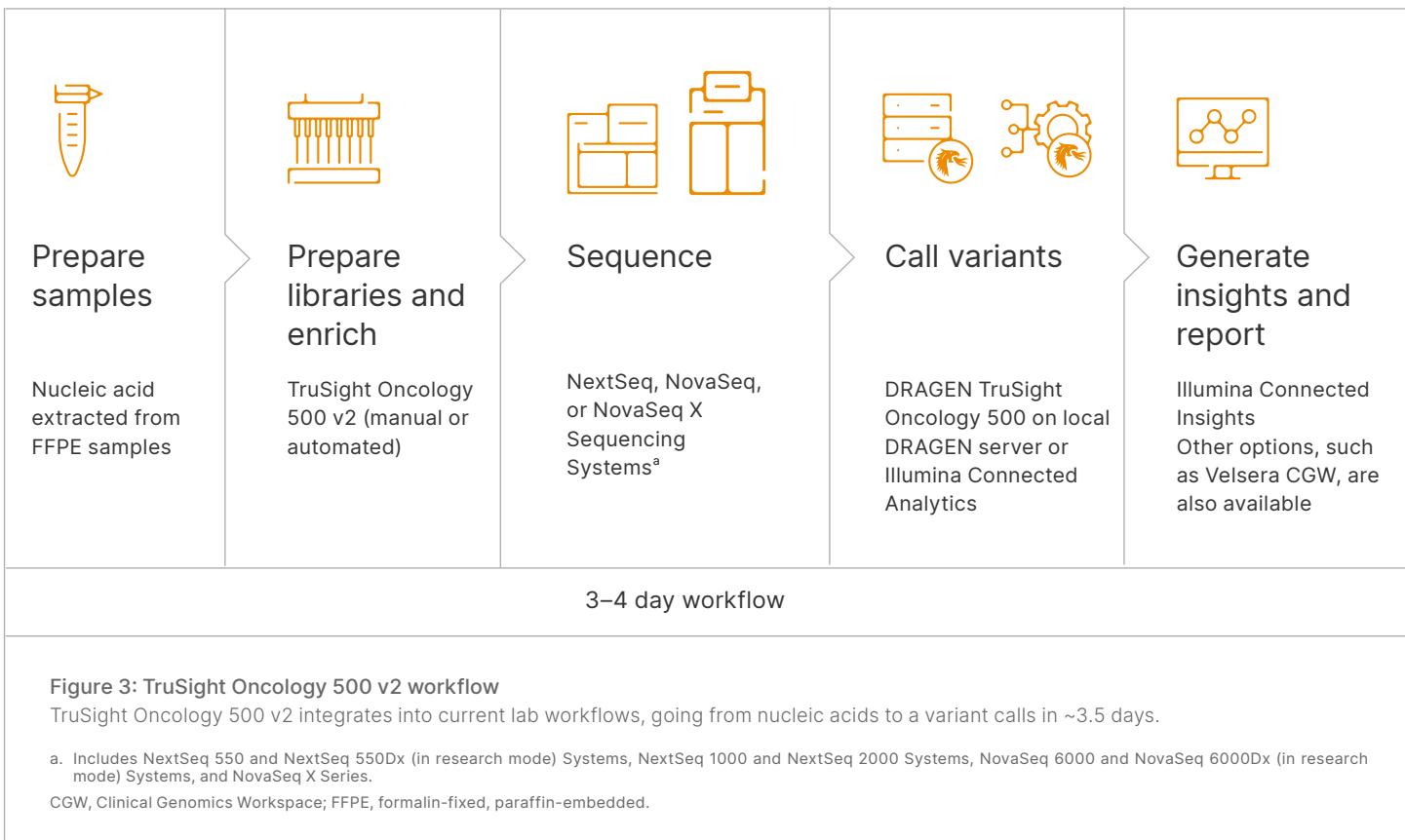


Table 3: End-to-end turnaround times by batch size

Configuration				Run time				
Batch size	System	Flow cell	Library prep workflow	Library prep	Sequencing	DRAGEN secondary analysis	Connected Insights case reporting	Total run time
8	NextSeq 550	High output	Manual	12 hr	22 hr	2 hr	10 min	~3.5 days
8	NextSeq 2000	P2	Manual	12 hr	19 hr	2 hr	10 min	~3.5 days
16	NovaSeq 6000	SP	Manual	13 hr	19 hr	4.5 hr	30 min	~3.5 days
32	NovaSeq X	1.5B	Manual	13 hr	20 hr	6.5 hr	1 hr	~3.5 days

## Fully incorporated HRD testing

With TruSight Oncology 500 v2, assessment of HRD can now be included for every sample. This feature reflects the growing importance of HRD as a cancer biomarker.<sup>6</sup> Homologous recombination repair (HRR) maintains genomic stability, and its impairment can lead to genomic instability and tumorigenesis.<sup>11</sup> Mutations in *BRCA1*, *BRCA2*, and other HRR-related genes are key drivers of HRD (Table 4).

Table 4: Genes involved in the HRR pathway<sup>12,13</sup>

ATM	CHEK2	RAD50
ATR	FANCA	RAD51
BARD1	FANCC	RAD51B
BRCA1	FANCI	RAD51C
BRCA2	FANCL	RAD51D
BRIP1	NBN	RAD54L
CDK12	PALB2	TP53
CHEK1	PTEN	

HRR, homologous recombination repair.

Aberrations that result in structural changes to chromosomes, or “genomic scars”, including loss of heterozygosity (LOH),<sup>14</sup> telomeric-allelic imbalance (TAI),<sup>15</sup> and large-scale state transitions (LST)<sup>16</sup> are indicators of the inability to repair DNA damage. These additional HRD biomarkers can be quantified and summed to generate a genomic instability score (GIS) (Table 5).

HRD assessment can now be incorporated into every sample, providing not only GIS, but also valuable genomic metrics, including:

- **CNV absolute copy number\***—aids in interpretation of gene deletions and amplifications, and is easier to use than fold-change CNVs
- **Tumor fraction**—provides an estimate of cancer cells in a tumor sample to inform sample quality
- **Tumor ploidy**—indicates the number of chromosome sets in a cell to inform data interpretation
- **Gene-level LOH<sup>†</sup>**—detects LOH events overlapping with panel genes to further aid in interpretation of copy number variants<sup>17</sup>

\* CNV absolute copy number is a beta feature that is not fully tested by Illumina.

† Gene-level LOH is a beta feature that is not fully tested by Illumina.

Table 5: The three genomic scars included in a GIS

Genomic scar	Description	
Loss of heterozygosity (LOH)	Loss of one allele from a gene pair, resulting in a homozygous region that may promote malignant growth if the remaining allele is impaired.	
Telomeric-allelic imbalance (TAI)	Unequal allele ratios at the chromosome ends (telomeres), where one chromosome carries a greater number of alleles than its pair.	
Large-scale state transitions (LST)	Structural breakpoints within a chromosomal pair, leading to inconsistencies between corresponding regions.	

## Determining HRD status

Several assays are available for measuring HRD status, each using different criteria.<sup>18</sup> Some assays assess only LOH to evaluate genomic instability. Increasing evidence suggests that assessing all three genomic scars (LOH, TAI, LST) improves detection of HRD-positive samples.<sup>19-21</sup> HRD status can also be defined by detection of pathogenic *BRCA1/2* mutations combined with a GIS. Unlike other commercial assays, the TruSight Oncology 500 v2 HRD solution enables in-house CGP, detection of *BRCA1/2* mutations, and determination of GIS status. The result is a highly sensitive, reliable assessment of HRD status and other cancer-associated genomic variants. HRD status is evaluated by TruSight Oncology 500 v2 using a proprietary algorithm powered by Myriad Genetics.

## From sample to results

### Start with DNA or RNA

The TruSight Oncology 500 v2 assay can use DNA or RNA extracted from the same sample as the input material. If using DNA, sample preparation starts with shearing the genomic DNA (gDNA). If starting from RNA, the first step is to reverse transcribe the sample into cDNA. Sequencing-ready libraries are prepared from sheared gDNA and cDNA simultaneously.

### Automate for efficiency

Illumina has partnered with leading liquid-handling manufacturers Beckman Coulter Life Sciences and Hamilton to produce fully automated workflows that support a range of throughput needs. Automated workflows can reduce hand-on time and increase walk-away time, enabling labs to save on labor costs and improve efficiency while achieving the same high-quality results as manual workflows.

### Add tags for analytical specificity

Unique molecular identifiers (UMIs)<sup>22</sup> are incorporated into gDNA or cDNA fragments prior to amplification to reduce error rates and enable detection of variants at low variant allele frequency (VAF).

### Enrich libraries to focus efforts

Hybrid-capture chemistry is a proven approach for target enrichment during library preparation, enabling the purification of selected regions from DNA- and

RNA-based libraries. This method uses biotinylated probes that hybridize to regions of interest, which are then captured with streptavidin-coated magnetic beads and eluted to enrich the library pool. Hybridization-based enrichment supports reliable analysis of specific genetic variants and enables sequencing of large gene panels, including those with more than 50 genes.

Compared with amplicon-based methods, hybrid-capture chemistry offers several advantages, including reduced artifacts and dropouts, and compatibility with a wide range of input types and quantities. It is also fusion agnostic, allowing for the detection and characterization of both known and novel fusions.

TruSight Oncology 500 v2 incorporates advances in this chemistry to streamline the workflow, reducing the number of hybridization steps from two to one and shortening library preparation to 1–2 days.

### Sequence 8–960 samples

TruSight Oncology 500 v2 offers flexible batching sizes ranging from 8 to 960 samples in a single assay. This is enabled by the availability of 384 unique indexes and flow cells that accommodate varying throughput levels. Each sample index performs consistently to produce sequencing metrics above quality control (QC) expectations.

### Analyze data

Variant calling for TruSight Oncology 500 v2 is available with DRAGEN secondary analysis in the cloud using Illumina Connected Analytics, now with data streaming and autolaunch capabilities. Analysis can also be performed on premises using a local DRAGEN server. The DRAGEN TruSight Oncology 500 v2 secondary analysis is straightforward to use and integrates easily with variant interpretation solutions. A fully automated analysis workflow can be configured to initiate secondary and tertiary analysis without requiring manual data transfers.

Illumina DRAGEN secondary analysis pipelines consist of sophisticated proprietary algorithms that remove errors, artifacts, and germline variants, resulting in highly accurate variant calling performance with an analytical specificity of > 99.9995%. This level of specificity is beneficial when it is critical to know the exact number of mutations per Mb, as in TMB evaluation with a tumor-only workflow.

 Read the [DRAGEN secondary analysis data sheet](#) to learn more about its variant calling features.

## Interpret Variants

TruSight Oncology 500 v2 can be used with multiple commercial variant interpretation solutions. Illumina Connected Insights offers the most integrated experience for DRAGEN TruSight Oncology 500 v2 data and enables a fully automated analysis workflow without 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. Visualization tools are optimized for TruSight Oncology 500 v2 data and include DNA and RNA coverage graphs ([Figure 4](#)), a genome view with B-allele ratio to leverage HRD panel data ([Figure 5](#)), and fusion plots that display breakpoints, reading frames, protein domains, supporting reads, and other key metrics for quality control and interpretation ([Figure 6](#)).

## Proven, reliable results

TruSight Oncology 500 v2 builds on the trusted performance of TruSight Oncology 500, delivering the same analytical rigor while enabling deeper genomic insights through expanded content and enhanced capabilities. TruSight Oncology 500 v2 demonstrates strong concordance with a reference standard. It also meets the same high performance expectations as TruSight Oncology 500 across key variant types, including MSI, TMB, CNVs, small variants, and fusions. In addition, TruSight Oncology 500 v2 provides increased DNA coverage, improved detection in challenging genomic regions, improved coverage of GC-rich and AT-rich regions, expanded CNV, and greater sensitivity at lower DNA and RNA input for FFPE samples.

### Increased panel coverage

TruSight Oncology 500 v2 now has higher DNA coverage than the original TruSight Oncology 500, with improved exon coverage ([Figure 7](#)).

### Coverage of GC-rich and AT-rich regions

TruSight Oncology 500 v2 has improved coverage of genomic regions that are difficult to amplify with PCR. This includes regions of interest such as the *TERT* promoter ([Figure 8](#)).

### Comprehensive detection of CNVs

Copy-number changes in several genes and tumor types have been associated with tumorigenesis. TruSight Oncology 500 v2 includes analysis of > 500 genes associated with CNVs and can call amplifications with a limit of detection at 1.8-fold change ([Table 6](#)).

### Highly sensitive variant detection from FFPE samples

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. This mechanism reduces sample dropouts in the presence of both natural allelic variations and sequence artifacts introduced from FFPE tissue samples. The assay can reproducibly detect variants in FFPE samples as low as 5% VAF ([Table 7](#)).

### Robust detection of fusions and splice variants

Cancer can arise from epigenetic changes, expression-level changes, or gene fusions that are undetectable by standard sequencing methods.<sup>23,24</sup> TruSight Oncology 500 v2 detects and characterizes fusions from the partner gene. An input of 40 ng RNA is recommended for use with TruSight Oncology 500 v2. However, fusion and splice variants may be detected with RNA input as low as 20 ng ([Table 8](#)).

### Detection of *BRCA* large rearrangements

A *BRCA* large rearrangement (LR) step in the DRAGEN TruSight Oncology 500 v2 analysis workflow enables exon-level CNV detection for *BRCA1* and *BRCA2* genes ([Table 9](#)).



Figure 4: Assessing gene and exon coverage for DNA and RNA with Illumina Connected Insights

Coverage across targeted genes and exons is displayed to reveal gaps that may impact variant detection and interpretation.

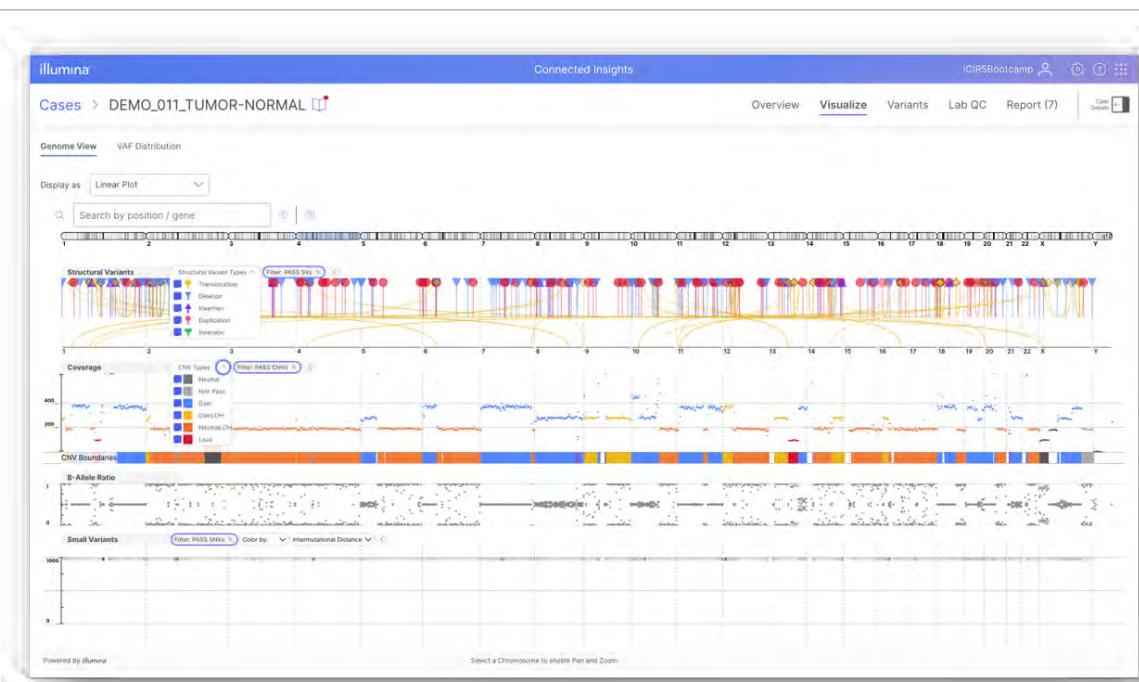


Figure 5: Genome-wide visualization of structural variants, copy number changes, and allelic imbalance

Illumina Connected Insights provides an integrated genome view that highlights large-scale alterations and genomic instability.

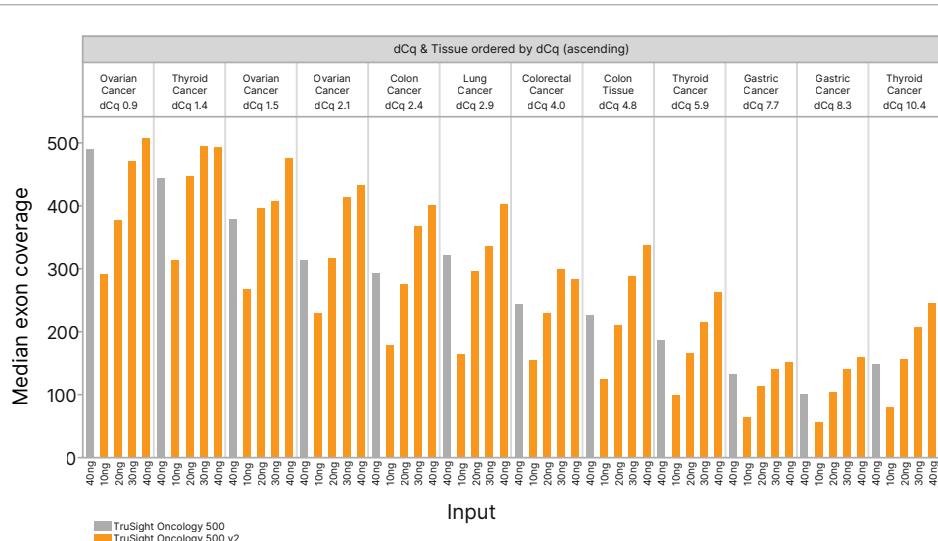
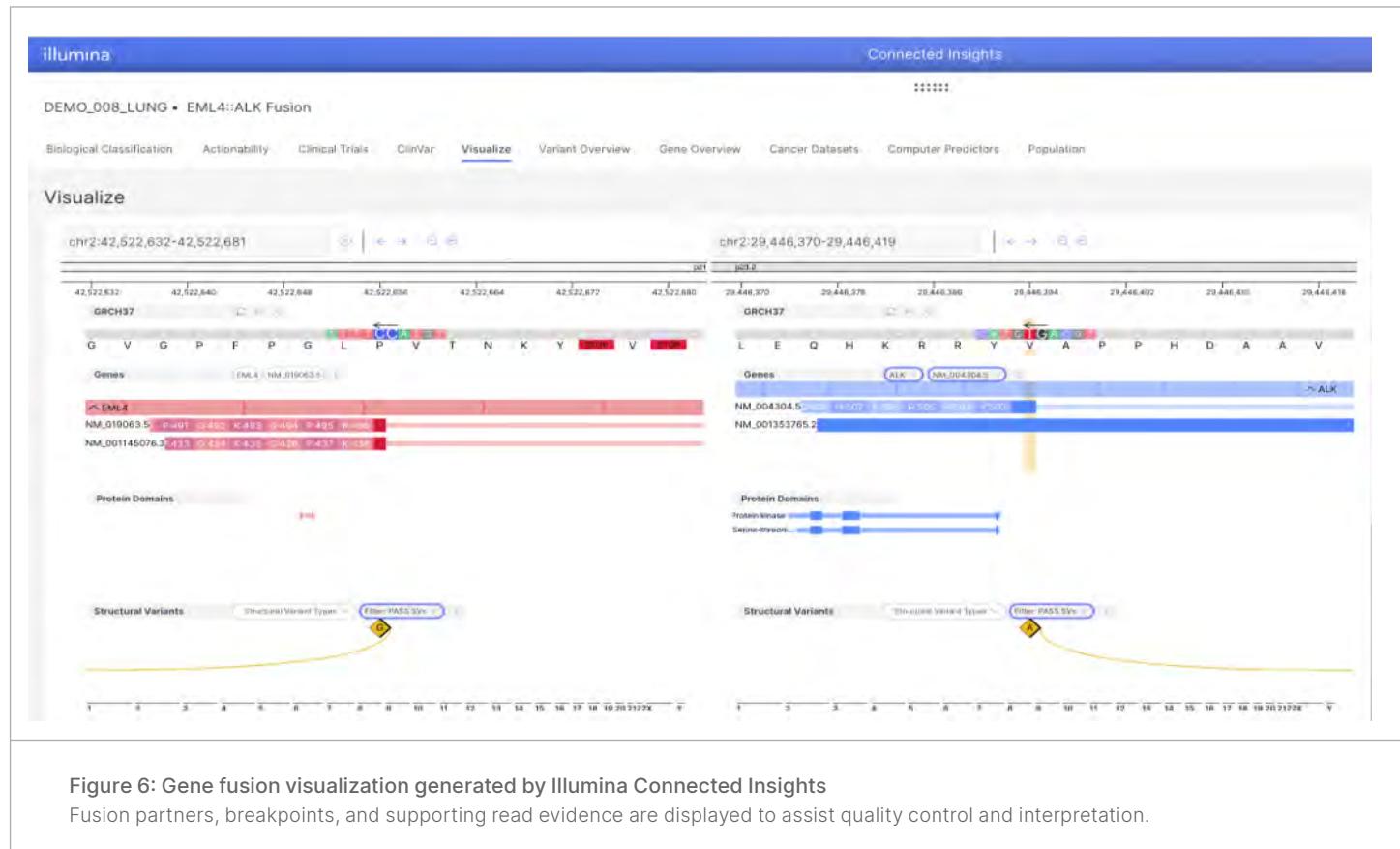


Figure 7: Improved exon coverage of TruSight Oncology 500 v2 across tissue types and input amounts

Median sequencing depth was evaluated for 13 tissue cancer types using TruSight Oncology 500 and TruSight Oncology 500 v2 with input quantities ranging from 10 ng to 40 ng, sorted by increasing dCq values. TruSight Oncology 500 v2 showed consistently improved exon coverage across most conditions, particularly at lower input levels.

dCq, differential quantification cycle.

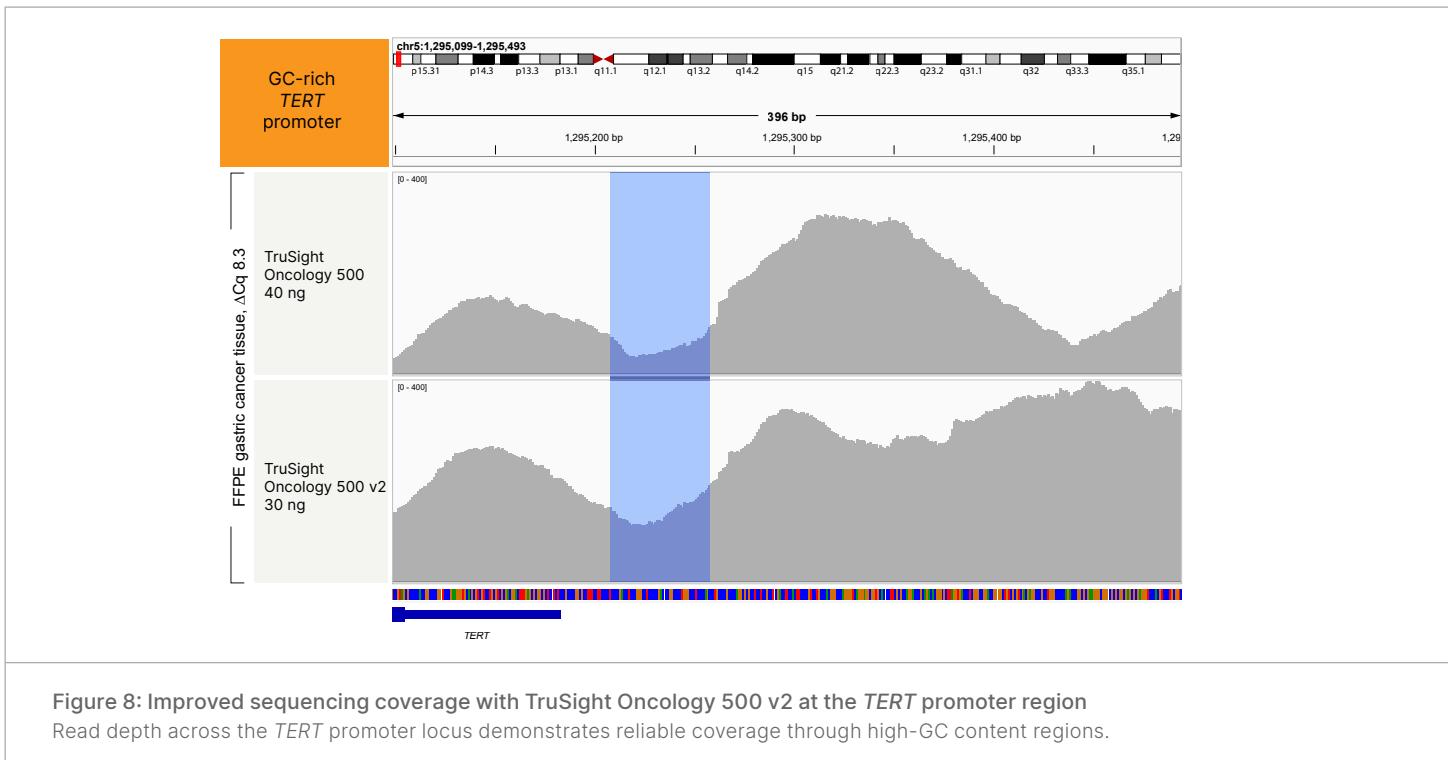


Table 6: CNV detection (mean fold change) by gene

Gene <sup>a</sup>	FFPE DNA dCq	TruSight	TruSight	TruSight Oncology 500 v2					
		Oncology 500	Oncology 500 HT	40 ng	40 ng	10 ng	20 ng	30 ng	40 ng
ALK	1.5	1.7	1.5	1.4	1.7	1.7	1.7	1.7	1.7
ALK	2.1	1.9	1.7	1.5	1.6	1.6	1.6	1.6	1.7
AKT2	10.4	1.6	1.6	1.5	1.5	1.5	1.5	1.5	1.5
AKT2	1.5	4.0	4.3	4.4	4.3	4.2	4.2	4.2	4.2
BRAF	0.9	1.6	1.7	1.5	1.5	1.5	1.5	1.5	1.6
CCND	0.9	1.6	1.7	1.7	1.6	1.6	1.6	1.6	1.6
CCNE1	1.5	3.7	3.9	3.9	3.9	4.0	4.0	3.0	
CCNE1	2.1	4.1	4.4	4.6	4.6	4.4	4.4	4.5	
ERBB2	2.1	17.5	17.4	17.4	17.5	17.4	17.5		17.5
JAK2	0.9	1.6	1.6	1.6	1.6	1.6	1.6		1.6
FGF9	≤ 0.9	3.6	3.6	4.0	3.8	3.8	3.8		3.8
KRAS	7.7	2.1	2.0	2.0 <sup>b</sup>	2.0	2.1	2.1		2.1
KRAS	8.3	3.5	3.6	3.8 <sup>b</sup>	3.9	3.9	4.0		4.0
KRAS	1.5	5.0	4.9	4.9	4.9	5.0	4.9		4.9
MET	≤ 9.0	1.6	1.5	1.5	1.6	1.6	1.6		1.6
MYCL	0.9	1.6	1.6	1.5	1.5	1.5	1.5		1.6
MYCN	2.1	1.6	1.6	1.7	1.7	1.7	1.7		1.7
PIK3CA	0.9	1.6	1.4	1.4	1.5	1.5	1.6		1.5
RPS6KB1	2.1	2.5	2.8	2.8	2.8	2.8	2.8		2.7

a. Some genes had multiple samples tested.

b. Library failed CNV QC.

Table 7: Variant detection for SNV, MNV, and indels for FFPE samples across input levels and reagent lots

FFPE sample	Variant	Variant type	C95		
			Lot 1	Lot 2	Max
Colon tissue	<i>BRAF</i> V600E	SNV	1.9%	3.7%	3.7%
Lung tissue	<i>CDKN2A</i> H83Y	SNV	1.0%	2.6%	2.6%
Lung tissue	<i>EGFR</i> L858R	SNV	1.9%	2.1%	2.1%
Cell lines	<i>EGFR</i> T790M	SNV	0.7%	1.1%	1.1%
Cell lines	<i>KRAS</i> A146T	SNV	4.8%	4.0%	4.8%
Lung tissue	<i>KRAS</i> G12V	SNV	1.6%	3.8%	3.8%
Cell lines	<i>KRAS</i> G13D	SNV	2.5%	3.7%	3.7%
Cell lines	<i>NRAS</i> G12V	SNV	1.5%	1.2%	1.5%
Colon tissue	<i>PIK3CA</i> E542K	SNV	3.6%	4.6%	4.6%
Colon tissue	<i>PIK3CA</i> H1047R	SNV	4.2%	4.3%	4.3%
Colon tissue	<i>KRAS</i> G13V (c.38_39delinsTT)	MNV	5.8%	6.8%	6.8%
Lung tissue	<i>AXIN2</i> G665Afs24	Deletion	7.8%	10.0%	10.0%
Colon tissue	<i>CREBBP</i> S1680del	Deletion	3.3%	3.1%	3.3%
Cell lines	<i>EGFR</i> ΔE746-A750	Deletion	0.7%	1.3%	1.3%
Colon tissue	<i>TP53</i> P191del	Deletion	3.5%	3.6%	3.6%
Breast tissue	<i>NF1</i> Y580Lfs8	Insertion	6.4%	7.2%	7.2%

FFPE tissue and cell line samples containing variants of interest were diluted across five test levels. At each level, 10 observations were generated using 30 ng input and two distinct reagent lots. C95 denotes the lowest mean VAF at which  $\geq 95\%$  detection was achieved for each lot. C95 Max indicates the higher (ie, more conservative) C95 value between the two lots.

FFPE, formalin fixed, paraffin embedded; MNV, multinucleotide variant; SNV, single nucleotide variant; VAF, variant allele frequency.

Table 8: Detection of fusion and splice variants at different RNA input levels

Assay		Supporting reads				
		TruSight Oncology 500		TruSight Oncology 500 v2		
RNA input		40 ng	20 ng	40 ng	60 ng	80 ng
Tissue	RNA fusion					
Liposarcoma	<i>TPM3-NTRK1</i>	37	54	105	150	197
Lung cancer	<i>KIF5B-RET</i>	19	17	50	67	197
Lung cancer	<i>EML4-ALK</i>	11	29	56	67	197
Fibrosarcoma	<i>ETV6-NTRK3</i>	431	1184	1790	1859	2117
Lung cancer	<i>FGFR3-TACC3</i>	170	534	820	960	1173
Breast cancer	<i>PVT1-MYC</i>	17	35	77	75	93
Lung cancer	<i>EML4-ALK</i>	15	18	32	51	67
Breast cancer	AR	41	19	38	58	68

Table 9: Detection of *BRCA* large rearrangements

<b><i>BRCA</i> 1/2 large rearrangements detected</b>	<b>TruSight Oncology 500 HRD VAF</b>		<b>TruSight Oncology 500 v2 VAF</b>		
	DNA input	40 ng	10 ng	20 ng	30 ng
<i>BRCA1</i> exon 13–23 loss	51.8%	47.0%	48.6%	50.7%	
<i>BRCA1</i> exon 20–23 loss	84.7%	82.2%	85.2%	86.2%	
<i>BRCA1</i> exon 2–3 loss	45.7%	39.3%	40.4%	46.8%	
<i>BRCA1</i> exon 2–22 loss	70.5%	64.3%	65.7%	67.0%	
<i>BRCA1</i> exon 2–3 loss	86.0%	86.8%	86.2%	86.0%	
<i>BRCA1</i> exon 2 loss	82.8%	79.8%	93.5%	89.6%	
<i>BRCA2</i> exon 25–27 loss	30.9%	Not detected	32.9%	38.7%	

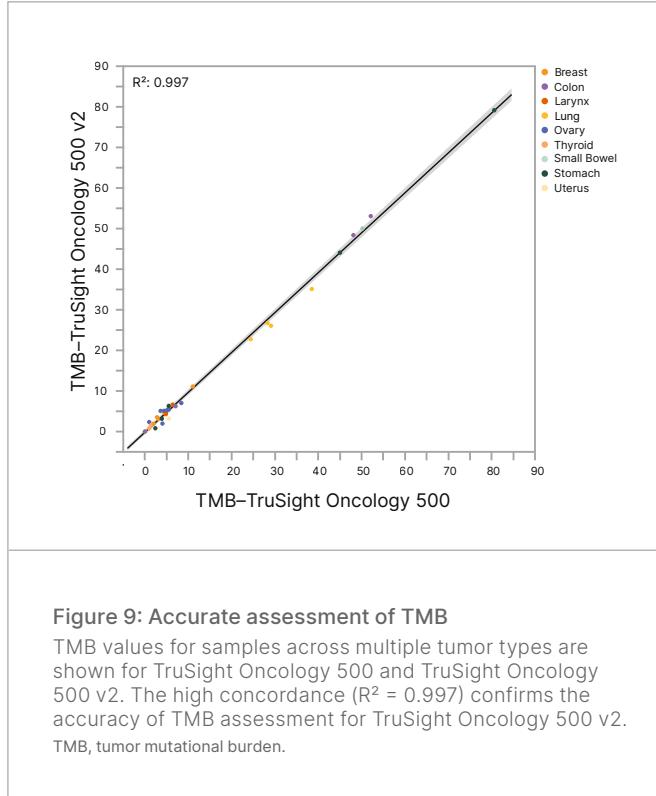
VAF, variant allele frequency.

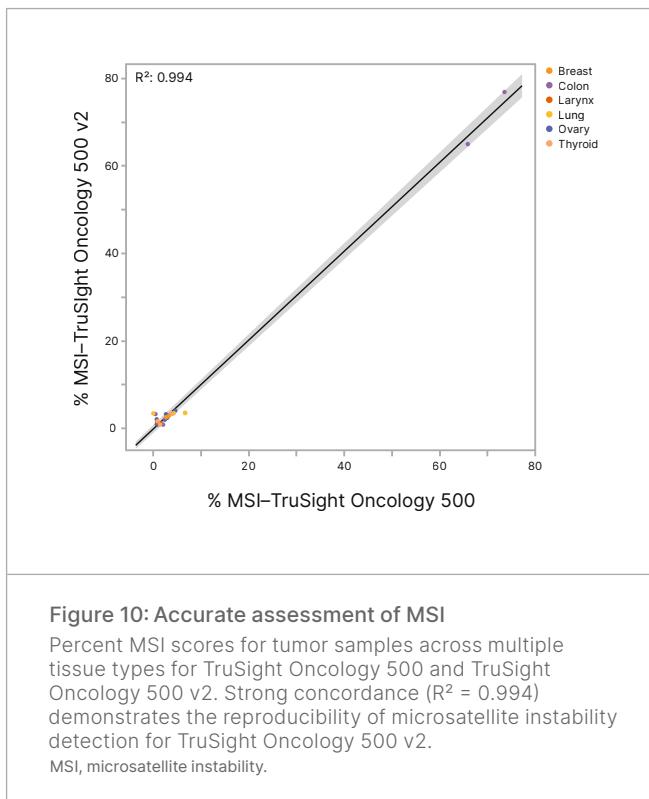
## IO gene signatures: TMB and MSI

TruSight Oncology 500 v2 is well suited to interrogate the immuno-oncology (IO) signatures TMB and MSI, which rely upon analysis of multiple genomic loci.

Obtaining a precise and reproducible TMB value at low mutation levels can be challenging with smaller panels. TruSight Oncology 500 v2 panels combine comprehensive genomic content with sophisticated informatics algorithms to provide accurate TMB estimation that is highly concordant with TruSight Oncology 500 results (Figure 9). The addition of UMIs during library preparation coupled with proprietary Illumina informatics reduces sequencing error rates by 10–20-fold.<sup>22</sup> Removing FFPE artifacts (such as deamination, oxidation assessment) enables analytical sensitivity as low as 5% VAF from low-quality DNA samples.

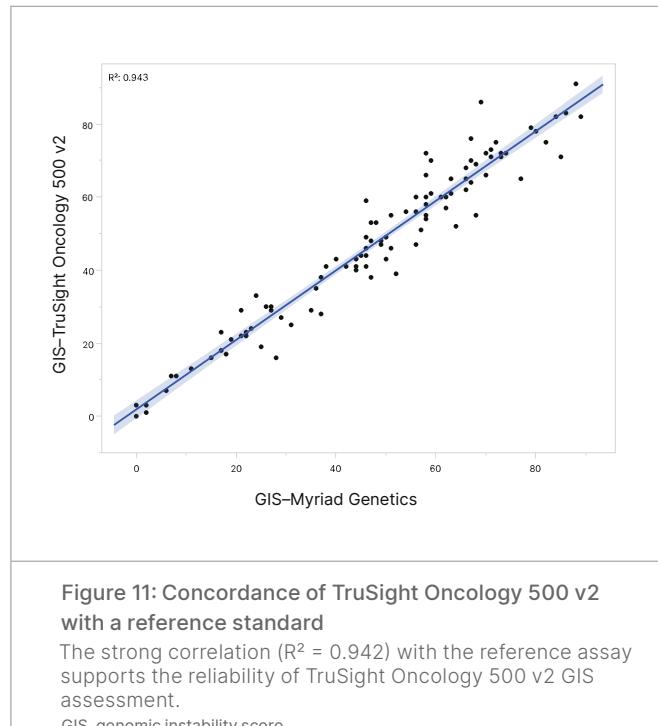
Traditionally, MSI status has been analyzed with PCR (MSI-PCR) and immunohistochemistry. While these methods deliver a qualitative result describing samples as either MSI-stable or MSI-high, NGS-based assessment with TruSight Oncology 500 v2 interrogates 130 homopolymer MSI marker sites to calculate an accurate quantitative score for MSI status.<sup>25</sup> MSI assessment by TruSight Oncology 500 v2 is highly concordant with MSI determined by TruSight Oncology 500 (Figure 10).





## Comprehensive assessment of HRD status

HRD status results from TruSight Oncology 500 v2 were compared to the current reference standard for HRD detection (Table 10). GIS determined using TruSight Oncology 500 v2 in 102 FFPE samples showed high concordance with the reference assay (Figure 11).



**Table 10: High concordance between TruSight Oncology 500 v2 HRD results and a reference standard**

Status (N = 102)	PPA	NPA	OPA
Overall HRD status	97.1 (66/68) 95% CI: 89.9–99.2	88.2 (30/34) 95% CI: 73.4–95.3	94.1 (96/102) 95% CI: 87.8–97.3
BRCA analysis	95.2 (40/42) 95% CI: 84.2–98.7	93.3 (56/60) 95% CI: 84.1–97.4	94.1 (96/102) 95% CI: 87.8–97.3
GIS	91.0 (61/67) 95% CI: 81.8–95.8	97.1 (34/35) 95% CI: 85.5–99.5	93.1 (95/102) 95% CI: 86.5–96.6

HRD status is defined as BRCA- or GIS-positive. Six discordant cases included three GIS results near the cutoff and three BRCA large rearrangement (LR) false positives below the limit of detection (LOD).

CI, confidence interval; GIS, genomic instability score; HRD, homologous recombination deficiency; NPA, negative percent agreement; OPA, overall percent agreement; PPA, positive percent agreement.

## Plan for the future

TruSight Oncology 500 v2 integrates easily into labs currently using NGS, enabling them to offer CGP capabilities without exploring a new technology. Bringing tumor assays in house also allows labs to keep sample and raw data. By consolidating multiple independent, single biomarker assays into one assay, labs can save sample, time, and money, while increasing the chances of identifying a positive biomarker. To further enhance detection capabilities, TruSight Oncology 500 v2 content is expanding, with the inclusion of additional biomarkers planned for a future date.

## Enhanced product attributes

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

- **Advanced change notification**—Illumina notifies laboratories six months in advance of any significant changes to products in the TruSight Oncology 500 portfolio
- **Certificate of Analysis**—Every TruSight Oncology 500 product includes a certificate of analysis (CoA) that confirms the product meets predefined release specifications and quality standards
- **Extended shelf life**—TruSight Oncology 500 v2 reagents have a minimum guaranteed shelf life of three months, with plans to extend guaranteed shelf life to six months, reducing the risk of expiration and supporting flexible testing schedules

## Summary

TruSight Oncology 500 v2 streamlines the detection of key cancer biomarkers with a newly optimized workflow that reduces turnaround time and input requirements while increasing sensitivity. Now with integrated HRD detection, the assay enables broad genomic coverage, capturing multiple variant types in a single test. Designed for scalability, it supports flexible batching and automated workflows across multiple Illumina sequencing platforms. TruSight Oncology 500 v2 delivers fast, high-accuracy variant calling, making CGP more efficient and accessible for cancer research.

**Learn more →**

[TruSight Oncology 500 v2](#)

[DRAGEN secondary analysis](#)

[Illumina Connected Analytics](#)

[Illumina Connected Insights](#)

## Appendix - DNA and RNA content included in TruSight Oncology 500 v2

ABL1	BCR	CHEK1	EPHA7	FGF8	GSK3B	IDH2	MAP3K1	NF2	PIK3CA	RAD51D	SMAD4	TGFB2
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	TMPPRSS2
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 <sup>a</sup>	CRLF2	ERCC1	FGF23	HIST1H1C	INSR	MAPK3	NOTCH1	PIK3R2	RARA	SMC3	TOP1
AKT3	BRCA2 <sup>a</sup>	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 <sup>b</sup>	
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	TGFB1	

a. Large rearrangements (exon-level CNVs) detected for BRCA1 and BRCA2.

b. Only the TERT promoter region is covered for variant calling.

Orange boxes indicate gene content for DNA and RNA known and novel fusions. Yellow boxes indicate gene content for DNA, RNA known and novel fusions, and RNA splice variants. Probes target at least 97% of the coding sequence for all genes in bold.

CNV calling is available for all genes except: DNAJB1, FANCF, FOXL2, HIST1H3A, HIST1H3C, HIST1H3D, HIST1H3E, HIST1H3F, HIST1H3G, HIST1H3H, HIST1H3I, HIST1H3J, HIST2H3A, HIST2H3C, HIST2H3D, HLA-A, HLA-B, HLA-C, KMT2B, KMT2D, TERC, TERT.

## Ordering information

Product	Catalog no.
TruSight Oncology 500 v2 DNA/RNA Kit (24 samples)	20130527
TruSight Oncology 500 v2 DNA Kit (48 samples)	20130528
TruSight Oncology 500 v2 DNA/RNA Automation Kit (32 samples)	20130529
TruSight Oncology 500 v2 DNA Automation Kit (64 samples)	20130530
TruSight Oncology 500 v2 DNA/RNA Automation Kit (96 samples)	20130532
TruSight Oncology 500 v2 DNA/RNA Kit plus Illumina Connected Insights (24 samples)	20138695
TruSight Oncology 500 v2 DNA Kit plus Illumina Connected Insights (48 samples)	20138696
TruSight Oncology 500 v2 DNA/RNA Kit plus Velsera (24 samples)	20138680
TruSight Oncology 500 v2 DNA Kit plus Velsera (48 samples)	20138681
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Illumina Connected Insights (32 samples)	20138698
TruSight Oncology 500 v2 DNA Automation Kit plus Illumina Connected Insights (64 samples)	20138773
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Illumina Connected Insights (96 samples)	20138774
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Velsera (32 samples)	20138682
TruSight Oncology 500 v2 DNA Automation Kit plus Velsera (64 samples)	20138683
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Velsera (96 samples)	20138685
NextSeq 550 sequencing reagent kits	
TruSight Oncology 500 v2 DNA/RNA Kit, NextSeq 550 (24 samples)	20130536
TruSight Oncology 500 v2 DNA Kit, NextSeq 550 (48 samples)	20130537
TruSight Oncology 500 v2 DNA/RNA Automation Kit, NextSeq 550 (32 samples)	20130542
TruSight Oncology 500 v2 DNA Automation Kit, NextSeq 550 (64 samples)	20130543
TruSight Oncology 500 v2 DNA/RNA Kit plus Illumina Connected Insights, NextSeq 550 (24 samples)	20138775
TruSight Oncology 500 v2 DNA Kit plus Illumina Connected Insights, NextSeq 550 (48 samples)	20138776
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Illumina Connected Insights, NextSeq 550 (32 samples)	20138777
TruSight Oncology 500 v2 DNA Automation Kit plus Illumina Connected Insights, NextSeq 550 (64 samples)	20138778
TruSight Oncology 500 v2 DNA/RNA Kit plus Velsera, NextSeq 550 (24 samples)	20138686
TruSight Oncology 500 v2 DNA Kit plus Velsera, NextSeq 550 (48 samples)	20138687
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Velsera, NextSeq 550 (32 samples)	20138688
TruSight Oncology 500 v2 DNA Automation Kit plus Velsera, NextSeq 550 (64 samples)	20138689

## Ordering information

Product	Catalog no.
NextSeq 1000 and 2000 sequencing reagent kits	
TruSight Oncology 500 v2 DNA/RNA Kit, NextSeq 1000/2000 P2 (24 samples)	20138676
TruSight Oncology 500 v2 DNA Kit, NextSeq 1000/2000 P2 (48 samples)	20138677
TruSight Oncology 500 v2 DNA/RNA Automation Kit, NextSeq 1000/2000 P2 (32 samples)	20138678
TruSight Oncology 500 v2 DNA Automation Kit, NextSeq 1000/2000 P2 (64 samples)	20138679
TruSight Oncology 500 v2 DNA/RNA Kit plus Illumina Connected Insights, NextSeq 1000/2000 P2 (24 samples)	20138779
TruSight Oncology 500 v2 DNA Kit plus Illumina Connected Insights, NextSeq 1000/2000 P2 (48 samples)	20138780
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Illumina Connected Insights, NextSeq 1000/2000 P2 (32 samples)	20138781
TruSight Oncology 500 v2 DNA Automation Kit plus Illumina Connected Insights, NextSeq 1000/2000 P2 (64 samples)	20138782
TruSight Oncology 500 v2 DNA/RNA Kit plus Velsera, NextSeq 1000/2000 P2 (24 samples)	20138690
TruSight Oncology 500 v2 DNA Kit plus Velsera, NextSeq 1000/2000 P2 (48 samples)	20138692
TruSight Oncology 500 v2 DNA/RNA Automation Kit plus Velsera, NextSeq 1000/2000 P2 (32 samples)	20138693
TruSight Oncology 500 v2 DNA Automation Kit plus Velsera, NextSeq 1000/2000 P2 (64 samples)	20138694
Index kits	
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)	20091660
Illumina DNA/RNA UD Indexes v3, Set A, Auto (96 Indexes 96 samples)	20141196
Illumina DNA/RNA UD Indexes v3, Set B, Auto (96 Indexes 96 samples)	20141197
Illumina DNA/RNA UD Indexes v3, Set C, Auto (96 Indexes 96 samples)	20141198
Illumina DNA/RNA UD Indexes v3, Set D, Auto (96 Indexes 96 samples)	20141199

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