

# TruSight™ Oncology 500 HRD

Uncover cancer genome insights with an all-in-one solution that enables CGP and HRD

- Assess genomic instability with a proprietary algorithm powered by Myriad Genetics
- Assay biomarkers across 500+ genes, including HRR causal variants and genomic signatures such as HRD, TMB, and MSI
- Increase efficiencies by bringing a highly accurate combined solution in house to enable CGP and HRD

Analysis powered by



## Introduction

As researchers continue to study the underlying genomics of cancer, they are uncovering broader molecular signatures occurring across cancer types. Homologous recombination deficiency (HRD) is one of these signatures, showing increasing importance in tumor biology for ovarian, breast, pancreatic, and prostate cancers.<sup>1</sup> However, HRD assessment may only be part of the story in these tumor types. Additional known and unknown genetic factors may driver tumor growth. For example, in ovarian cancer *BRCA1* and *BRCA2* mutations only comprise ~20% of high-grade serous ovarian cancer (HGSOC) (Figure 1).<sup>2</sup> Other genetic mutations may be present, including gene variants and molecular signatures such as tumor mutational burden (TMB) and microsatellite instability (MSI). Identifying additional possible contributors to tumor growth may provide researchers valuable information upfront.

To obtain a comprehensive view of the tumor genetics, additional information is required. Iterative, single-gene testing or small multigene panels can be used. However, these approaches yield less information per assay and require additional sample and time. Another option for understanding the genetic underpinnings of a tumor is comprehensive genomic profiling (CGP). CGP is a next-generation sequencing (NGS)-based method that enables simultaneous assessment of hundreds of biomarkers in a single test, from a single sample, maximizing the ability to find relevant alterations.

TruSight Oncology 500 HRD is an NGS-based research assay that harnesses the power of proven Illumina NGS technology and the Myriad Genetics GIS algorithm to enable CGP and HRD assessment. With one sample and one workflow, the in-house TruSight Oncology 500 HRD assay (Table 1, Table 2) provides labs with accurate, sensitive information on 500+ genes and genomic signatures that can help reveal the genomic nature of a tumor and unlock insights.

## About HRD

HRD is a complex genomic signature resulting from a cell's inability to repair double-stranded DNA breaks using the homologous recombination repair (HRR) pathway.

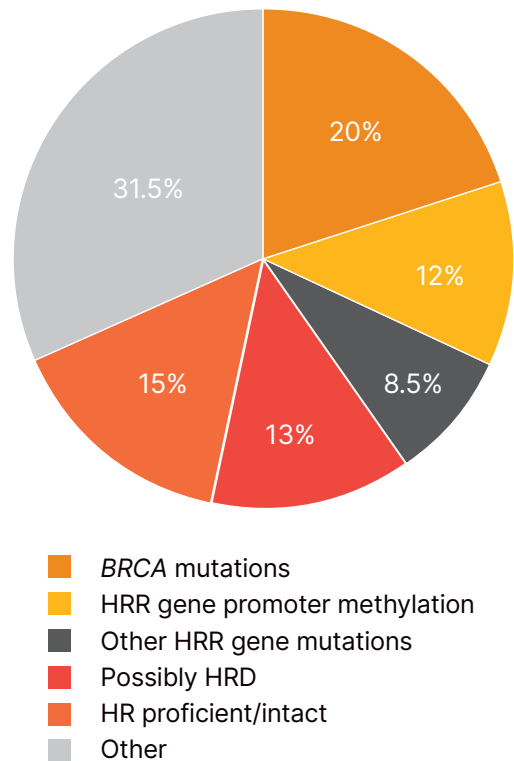


Figure 1: Tumors may harbor genetic alterations beyond *BRCA* mutations and genomic instability—While approximately 50% of HGSOC samples have a positive HRD status, there are a significant number of tumors attributed to factors beyond *BRCA* mutations and genomic scarring.<sup>2</sup>

The ability to repair DNA damage is essential for maintaining genomic stability and cellular functions, ensuring chromosomal integrity and cell viability. The HRR pathway is mediated by multiple genes, with *BRCA1* and *BRCA2* playing key roles (Table 3).<sup>2-6</sup> If the HRR pathway is impaired, double-stranded breaks are either not repaired or repaired using the error-prone nonhomologous end joining (NHEJ) pathway. These alternatives can result in genomic instability, in the form of genomic scars, leading to tumorigenesis.<sup>7</sup>

## Genomic scarring and GIS

Genomic scars are aberrations that result in structural changes in the chromosomes. The most relevant genomic scars are loss of heterozygosity (LOH),<sup>8</sup> telomeric-allelic imbalance (TAI),<sup>9</sup> and large-scale state transitions (LST)<sup>10</sup> (Table 4). When measured together, the three genomic scars produce a genomic instability score (GIS) that can be used as an indicator of HRD status.

Table 1: TruSight Oncology 500 HRD: CGP + HRD content

Feature	Description
<b>Enable CGP</b>	
Gene count	DNA: 523, RNA: 55
Panel size	1.94 Mb DNA, 358 kb RNA
Guideline coverage	Key guidelines for multiple solid tumor types
Clinical trial coverage	> 1000
Immuno-oncology biomarkers	TMB, MSI
Pan-cancer biomarkers	NTRK1, NTRK2, NTRK3
Analysis software	DRAGEN TruSight Oncology 500
<b>HRD status</b>	
No. probes	~25K
Cross ethnicity coverage	EAS, EUR, AFR, AMR, SAS <sup>a</sup>
<i>BRCA1/BRCA2</i> coverage	Small variants and large rearrangement variants
Genomic scars assessed	LOH, TAI, LST
GIS	Numerical score out of 100
GIS algorithm	Powered by Myriad Genetics
Analysis software	DRAGEN TruSight Oncology 500
a. AFR, Africa; AMR, ad-mixed American; EAS, East Asian; EUR, European; SAS, South Asian	

Table 2: TruSight Oncology 500 HRD: Assay details

Feature	Description
Input requirement	DNA: 40 ng
	RNA: 40 ng
	FFPE: Minimum recommendation of 2 mm <sup>3</sup> from FFPE tissue samples
Sample throughput (TruSight Oncology 500 + TruSight Oncology 500 HRD)	8 samples per run (NextSeq 550 System or NextSeq 550Dx System (research mode))
	16 samples per run (NovaSeq 6000 System)
Hands-on time	~10.5 hr
Total assay time	4–5 days from nucleic acid to variant report
Sequencing system	NextSeq 550 System or NextSeq 550Dx System (research mode) or NovaSeq 6000 System (SP flow cell)
Sequence run time	24 hr (NextSeq 550 High Output Kit)
	19 hr (NovaSeq 6000 SP)
Sequence run	2 × 101 cycles
Limit of detection	<b>HRD</b> GIS: 32% tumor content
	<b>CGP</b> Small variants: 5% VAF Fusions: 5 copies per ng RNA CNVs: 2.2× fold-change BRCA large rearrangements (≥ 3 exons): 43% VAF BRCA large rearrangements (< 3 exons): 50% VAF
	Analytical sensitivity TruSight Oncology 500: > 96% (for all variant types at 5% VAF)
Analytical specificity	GIS: 100% <sup>a</sup> TruSight Oncology 500: 99.9998% (for SNV detection)
a. Internal limit of blank study results from normal ovarian samples	

## Determining HRD status




HRD status can be determined by evaluating the presence of causal genes (*BRCA* and other HRR genes) and/or the effect of genomic scarring. Currently, there are several assays available for measuring HRD status, each with its own criteria.<sup>11</sup> Some assays only assess %LOH to determine genomic instability. There is increasing evidence that assessing all three genomic scars (LOH, TAI, LST) may maximize identification of HRD+ samples.<sup>12-14</sup> Unlike other commercial assays, the TruSight Oncology 500 HRD solution enables in-house CGP and evaluation of all three genomic scars.\* The result is a highly sensitive, reliable assessment of HRD status and other cancer-associated genomic variants potentially present in a sample.

Table 3: Genes involved in the HRR pathway<sup>2,6</sup>

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

\* Genomic scarring is evaluated using a proprietary algorithm powered by Myriad Genetics.

Table 4: The three genomic scars included in a GIS

Genomic scar	Description	
Loss of heterozygosity (LOH)	One of the two alleles for a gene is lost, creating a homozygous cell. This may result in malignant cell growth if the remaining allele does not function properly.	
Telomeric-allelic imbalance (TAI)	The allele ratios at the end of the chromosomes (telomere) in a pair do not match. That is, one chromosome has a greater number of alleles than the other.	
Large-scale state transitions (LST)	Breakpoints between regions of the chromosome resulting in discrepancies within the chromosome pair.	

## Comprehensive content

Content for TruSight Oncology 500 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, large rearrangements in BRCA genes, and complex immuno-oncology genomic signatures, such as microsatellite instability (MSI) and tumor mutational burden (TMB).

TruSight Oncology 500 HRD also includes ~25K genome-wide probes specifically designed to assess for genomic scars across a broad range of ethnicities. The number of SNPs needed for GIS assessment was determined using an *in silico* simulation and the SNPs were selected using data from the 1000 Genomes Project.

## Streamlined workflow

Enabling CGP with HRD assessment in house is simplified with the availability of a comprehensive, streamlined workflow that spans from sample input to final variant report (Figure 2). For maximum efficiency, the HRD assay is optimized to run with the standard TruSight Oncology 500 assay. No additional time is required. Ready-to-use library preparation kits, straightforward methods, and accurate, rapid variant calling pipelines enable a workflow that can be completed in as few as four days.

### Start with DNA or RNA and DNA

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

 Visit [www.illumina.com/tso500](http://www.illumina.com/tso500) to view a gene list

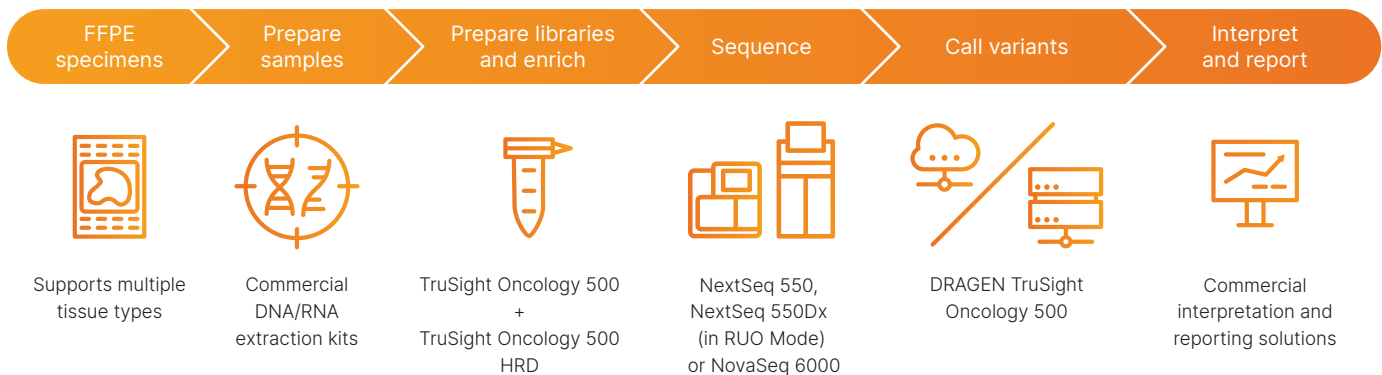


Figure 2: Streamlined TruSight Oncology HRD workflow—TruSight Oncology 500 HRD integrates into current lab workflows, going from nucleic acids to variant calls in four days.

## Add tags for analytical specificity

During library preparation, unique molecular identifiers (UMIs)<sup>15</sup> are added to the gDNA fragments. These UMIs enable detection of variants at low variant allele frequency (VAF) while simultaneously suppressing errors, providing high analytical specificity.

## Enrich libraries to focus efforts

Library preparation is based on proven hybrid-capture chemistry to purify selected targets from DNA- and RNA-based libraries. DNA enrichment with the HRD probes occurs on the same plate at the same time as the TruSight Oncology 500 enrichment. Biotinylated probes hybridize to regions of interest, which are pulled down using streptavidin-coated magnetic beads, and then eluted to enrich the library pool. The hybrid-capture method is highly sensitive and can accurately characterize gene fusions with both known and novel partners. TruSight Oncology 500 libraries and HRD libraries are pooled prior to sequencing.

## Sequence samples

The pooled TruSight Oncology 500 and HRD libraries are sequenced on the NextSeq™ 550, NextSeq 550Dx,<sup>†</sup> or NovaSeq™ 6000 System. The NextSeq systems offer eight samples per run, while the NovaSeq system enables 16 samples per run on an SP flow cell.<sup>‡</sup> Because the TruSight Oncology 500 and HRD libraries are pooled prior to sequencing, sample throughput is maintained. Each sample index performs consistently to produce sequencing metrics above quality control (QC) expectations.

## Fast, accurate analysis

Variant calling for TruSight Oncology 500 HRD is available through the DRAGEN™ TruSight Oncology 500 v2 Analysis Software using accelerated, fully integrated bioinformatics algorithms to ensure optimal assay performance. In addition to faster analysis time, the v2 version incorporates a highly sophisticated HRD pipeline that includes a pro-

prietary GIS algorithm powered by Myriad Genetics to ensure accurate results and outputs a comprehensive GIS. The DRAGEN TruSight Oncology 500 v2 Analysis Software is also able to call *BRCA* large rearrangement (LR) variants.

DRAGEN analysis can be run on a local DRAGEN server or using a cloud-based version run via Illumina Connected Analytics (ICA) (coming soon). All versions take advantage of sophisticated, proprietary algorithms that remove errors and artifacts.

The result is the ability to detect mutations across 500+ genes at 5% variant allele frequency (VAF) for small variants, with > 96% analytical sensitivity and > 99.9998% analytical specificity (Table 2 and Table 5). This level of specificity is particularly beneficial when it is critical to know the exact number of mutations per Mb, as in TMB evaluation with a tumor-only workflow. DNA variant data analyzed with the TruSight Oncology 500 Local App and DRAGEN TruSight Oncology 500 pipeline show concordant results; however, analysis with the DRAGEN pipeline is completed 2–4× faster than with the local app, reducing the time to final results.

Table 5: High-accuracy detection of *BRCA* small variants

Variant	VAF	Detection rate
BRCA2:N289H	5%	100%
BRCA2:N991D	5%	100%
BRCA1:S1613G	5%	100%
BRCA1:K1183R	5%	100%
BRCA1:K820E	5%	100%
BRCA1:D435Y	5%	100%

Six *BRCA* small variants originally at 7.5% VAF in the *BRCA* Somatic Multiplex I reference material (Horizon Discovery) were diluted to 5% VAF. Detection rate was evaluated using the TruSight Oncology 500 HRD assay.

<sup>†</sup> In Research Mode only

<sup>‡</sup> Achieving 16 samples per run using the DNA/RNA kit requires use of the NovaSeq Xp workflow

## Reproducible, trustworthy results

To demonstrate the high-quality results achieved with TruSight Oncology 500 HRD, Illumina performed various comparison studies with the current reference standard test for HRD detection. Data across a large reference standard cohort were compared to data from the same samples run using TruSight Oncology 500 HRD. For all samples, data were highly concordant, with an  $R^2$  value of 0.98 for GIS (Figure 3, Table 6).

To confirm that the addition of HRD testing did not impact variant calling, results from the TruSight Oncology 500 HRD assay were compared to those generated using TruSight Oncology 500. Data from various sample types and for various variant types showed high concordance (Figure 4, Table 7).

Table 6: High agreement rates for HRD status between TruSight Oncology 500 HRD and a reference standard

	PPA, % (95% CI)	NPA, % (95% CI)	OPA, % (95% CI)
Overall HRD status (N = 194)	95.2 (89.2-97.9)	96.8 (91.0-98.9)	96.0 (92.2-91.9)
BRCA analysis (N = 197)	92.9 (83.0-97.2)	98.6 (95.0-99.6)	96.9 (93.5-98.6)
HRD GIS (N = 204)	95.1 (89.1-97.9)	97.1 (91.9-99.0)	96.1 (92.6-98.0)

PPA, positive percent agreement; NPA, negative percent agreement; OPA, overall percent agreement (Data on file reference)

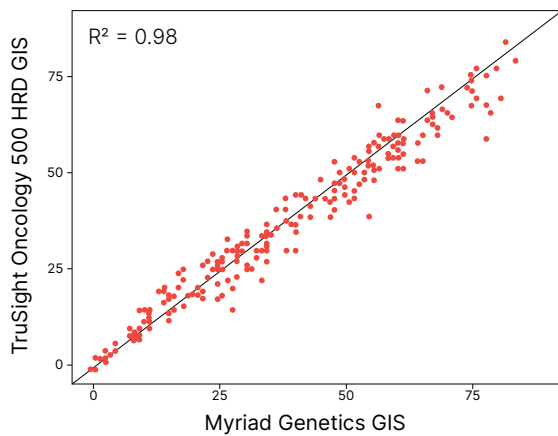


Figure 3: TruSight Oncology 500 HRD GIS is concordant to the Myriad Genetics GIS—For TruSight Oncology 500 HRD, forty nanograms of DNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples was used as assay input. For each sample, the DNA library was split into two hybridization reactions, one with TruSight Oncology 500 probes and the other with HRD probes. Both libraries were pooled for sequencing with eight samples per run on a NextSeq 550 System. Analysis was performed using the DRAGEN TruSight Oncology 500 v2 Analysis Software. Samples were also tested with a reference assay as the orthogonal test.

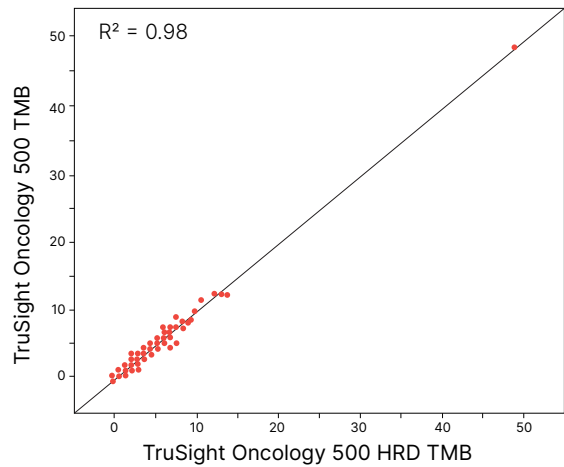


Figure 4: High-concordance TMB results between TruSight Oncology 500 and TruSight Oncology 500 HRD—A set of 125 ovarian cancer samples was sequenced using the TruSight Oncology 500 assay and the TruSight Oncology 500 HRD assay.



Table 7: Concordance results between TruSight Oncology 500 and TruSight Oncology 500 HRD by variant type

Variant type	Concordance
Small variants	PPA = 99.43% NPA = 99.99% OPA = 99.99%
CNVs	PPA = 96.79% NPA = 99.65% OPA = 99.40%
MSI	OPA = 100%

## Bring HRD testing in house

TruSight Oncology 500 HRD integrates easily into labs currently using NGS, enabling them to offer CGP combined with HRD assessment, without exploring an entirely new workflow or technology. Bringing tumor assays in house allows labs to keep sample and raw data, positively affecting turnaround time and sample coordination. By consolidating multiple independent assays into one assay, labs can save sample, time, and money, while increasing the chances of identifying a positive biomarker.

## Summary

TruSight Oncology 500 HRD provides labs an accurate in-house solution that enables CGP and HRD assessment. The HRD testing yields a comprehensive GIS that includes all three critical genomic scars with performance that is on par with the current reference standard test. But, HRD may not tell the whole tumor story. Combining HRD assessment with a comprehensive assay that reports on 500+ genes maximizes information on relevant biomarkers and genomic signatures obtained from a single sample in an efficient, all-in-one workflow.

## Learn more

TruSight Oncology 500 HRD, [www.illumina.com/tso500](http://www.illumina.com/tso500)

DRAGEN Bio-IT Platform, [illumina.com/products/by-type/informatics-products/dragen-bio-it-platform](http://illumina.com/products/by-type/informatics-products/dragen-bio-it-platform)

### Ordering information

Product	Catalog no.
TruSight Oncology 500 HRD <sup>a</sup> (24 samples)	20076480
TruSight Oncology 500	varies <sup>b</sup>
DRAGEN TruSight Oncology 500 HRD Analysis Software, On-Premise <sup>a</sup>	20073738
DRAGEN TruSight Oncology 500 HRD Analysis Software, Cloud <sup>a</sup>	20073740

a. Not available for sale in the United States or Japan

b. Visit [illumina.com/tso500](http://illumina.com/tso500) for a complete list of TruSight Oncology 500 kits

## References

1. Yamamoto H, Hirasawa A. [Homologous Recombination Deficiencies and Hereditary Tumors](#). *Int J Mol Sci*. 2021;23(1):348. Published 2021 Dec 29. doi:10.3390/ijms23010348
2. Konstantinopoulos PA, Ceccaldi R, Shapiro GI, D'Andrea AD. [Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer](#). *Cancer Discov*. 2015;5(11):1137-1154. doi:10.1158/2159-8290.CD-15-0714
3. Prakash R, Zhang Y, Feng W, Jasin M. [Homologous recombination and human health: the roles of BRCA1, BRCA2, and associated proteins](#). *Cold Spring Harb Perspect Biol*. 2015;7(4):a016600. Published 2015 Apr 1. doi:10.1101/cshperspect.a016600
4. Moynahan ME, Jasin M. [Mitotic homologous recombination maintains genomic stability and suppresses tumorigenesis](#). *Nat Rev Mol Cell Biol*. 2010;11(3):196-207. doi:10.1038/nrm2851
5. King MC. ["The race" to clone BRCA1](#). *Science*. 2014;343(6178):1462-1465. doi:10.1126/science.1251900
6. da Cunha Colombo Bonadio RR, Fogace RN, Miranda VC, Diz MDPE. [Homologous recombination deficiency in ovarian](#)



- cancer: a review of its epidemiology and management. *Clinics* (Sao Paulo). 2018;73(suppl 1):e450s. Published 2018 Aug 20. doi:10.6061/clinics/2018/e450s
7. O'Connor MJ. Targeting the DNA Damage Response in Cancer. *Mol Cell*. 2015;60(4):547-560. doi:10.1016/j.molcel.2015.10.040
  8. Abkevich V, Timms KM, Hennessy BT, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer*. 2012;107(10):1776-1782. doi:10.1038/bjc.2012.451
  9. Birkbak NJ, Wang ZC, Kim JY, et al. Telomeric allelic imbalance indicates defective DNA repair and sensitivity to DNA-damaging agents [published correction appears in *Cancer Discov*. 2013 Aug;3(8):952]. *Cancer Discov*. 2012;2(4):366-375. doi:10.1158/2159-8290.CD-11-0206
  10. Popova T, Manié E, Rieunier G, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res*. 2012;72(21):5454-5462. doi:10.1158/0008-5472.CAN-12-1470
  11. Stewart MD, Vega DM, Arend RC, et al. Homologous Recombination Deficiency: Concepts, Definitions, and Assays. *The Oncologist*. 2022;27(3):1. doi:10.1093/oncolo/oyab053
  12. Timms KM, Abkevich V, Hughes E, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. *Breast Cancer Res*. 2014;16(6):475. Published 2014 Dec 5. doi:10.1186/s13058-014-0475-x
  13. Marquard AM, Eklund AC, Joshi T, et al. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. *Biomark Res*. 2015;3(9). doi:10.1186/s40364-015-0033-4
  14. Timms KM Comparison of genomic instability test scores used for predicting PARP activity in ovarian cancer [abstract]. In: 2020 ASCO Annual Meeting; May 29-31, 2020; Virtual.
  15. Illumina. TruSight Oncology UMI Reagents technical note. <https://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/trusight-oncology-umi-reagents-datasheet-1000000050425.pdf>. Published 2018. Accessed March 30, 2022.

# illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel  
techsupport@illumina.com | www.illumina.com

© 2022 Illumina, Inc. All rights reserved. All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see [www.illumina.com/company/legal.html](http://www.illumina.com/company/legal.html).  
M-GL-00748 v1.0