DATA SHEET

TruSight™ Hereditary Cancer Panel

NGS panel for cancer research featuring 113 genes associated with genetic risk

- Evaluate germline mutations associated with cancer risk using comprehensive, expert-selected panel content
- Enrich and prepare sequencing-ready libraries in 6.5 hours, with only 2 hours hands-on time
- Achieve excellent coverage uniformity for highly accurate detection of SNVs, indels, and CNVs
- Adjust throughput and sequence 2–256 samples per run on Illumina benchtop sequencing systems

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Introduction

Genetic variants play an important role in determining cancer predisposition. The TruSight Hereditary Cancer Panel enables researchers to perform a comprehensive evaluation of the genes in which these variants are located. Developed in collaboration with experts in cancer genomics, the TruSight Hereditary Cancer Panel is a targeted sequencing panel designed to assess germline mutations across 113 genes and 125 single nucleotide polymorphisms (SNPs) for variant identification and polygenic risk scoring.

The assay uses predesigned, ready-to-use oligo probes that cover all exonic regions and 20 bp of flanking intronic regions for each targeted gene. Libraries are prepared using hybrid–capture chemistry integrated with Illumina DNA Prep with Enrichment.* Illumina DNA Prep with Enrichment uses an innovative bead-based chemistry with a simplified, single hybridization step for fast and efficient library preparation. Illumina DNA Prep with Enrichment is compatible with all Illumina benchtop sequencing systems, offering flexibility in experimental design across a wide range of sample throughput (Table 1).[1] Combining the speed of Illumina DNA Prep with Enrichment with the MiSeq™ System, the entire workflow (Figure 1), from sample to data, can be completed in 48 hours.

* Illumina DNA Prep was formerly known as Nextera™ DNA Flex Library Prep kit. The two kits use the same tagmentation chemistry and have identical product performance specifications and kit configurations.

Table 1: TruSight Hereditary Cancer Panel specifications

Parameter	Details
Supported sequencing systems	iSeq™ 100 System, MiniSeq™ System, MiSeq System, MiSeqDx System (in research mode), NextSeq™ 550 System, NextSeq 550Dx System (in research mode)
Panel size	403 kb, 113 genes (covering all exons), 125 SNPs (48 ID SNPs and 77 SNPs for polygenic risk score)
No. of probes	10,341 oligo probes
Sample type	Genomic DNA, blood[a], or saliva[a]
DNA input	50–1000 ng DNA
Total assay time	48 hours from DNA to data
Library prep time	6.5 hrs total time, 2 hrs hands-on time
Sample throughput	384 indexes available for variable throughput from 2–256 samples per run at average coverage of 300× (minimum coverage 100×)
Samples per tube	8 enrichments (up to 12 samples per enrichment)

a. Extraction directly from blood or saliva requires use of the Flex Lysis Reagent Kit (Illumina, Catalog no. 20018706).

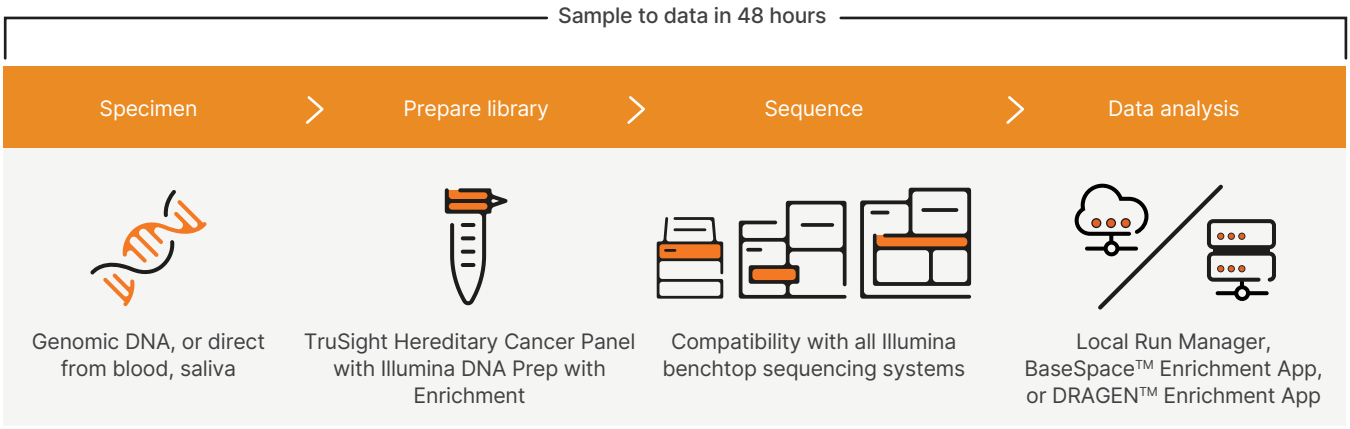

Figure 1: Fast, flexible NGS workflow— The TruSight Hereditary Cancer Panel uses Illumina DNA Prep with Enrichment library prep chemistry, which integrates library preparation and enrichment steps. A fast, streamlined, and optimized workflow delivers fully enriched libraries in just 6.5 hours. TruSight Hereditary Cancer is compatible with the iSeq 100, MiniSeq, MiSeq Series, and NextSeq Systems.

Flexibility of throughput with Illumina sequencing systems

The TruSight Hereditary Cancer Panel is compatible with multiple Illumina sequencing systems, providing flexibility and control over experimental design. Users can select instruments or reagent kits according to laboratory needs. Sample throughput can range from 2–256 samples per run (Table 2).

Comprehensive content design

The TruSight Hereditary Cancer Panel includes an extensive list of genes commonly associated with hereditary predisposition to breast, colon, ovarian, and gastric cancers (Figure 2). The content was developed with input and feedback from key opinion leaders on genetic risk assessment from Germany, France, and the United Kingdom. The panel includes 10,341 probes that target 113 genes related to cancer predisposition (Table 3), and evaluated on population studies of cases vs. controls. Also included are 48 SNPs for identity and gender determination purposes, and 77 SNPs for BOADICEA polygenic risk score.[2,3] Analysis enables the detection of single-nucleotide variants (SNVs), insertions/deletions (indels), and copy-number variants (CNVs) in a single assay (Table 4, Table 5).

Table 3: TruSight Hereditary Cancer Panel gene content

ACD	*DIS3L2*	*GREM1*	*PIK3CA*	*SDHD*
AIP	*EPCAM*	*HOXB13*	*PMS2*	*SLX4*
AKT1	*ERCC1*	*KIF1B*	*POLD1*	*SMAD4*
APC	*ERCC2*	*KIT*	*POLE*	*SMARCA4*
ATM	*ERCC3*	*LZTR1*	*POT1*	*SMARCB1*
BAP1	*ERCC4*	*MAX*	*PRKAR1A*	*SMARCE1*
BARD1	*ERCC5*	*MEN1*	*PTCH1*	*SPINK1*
BLM	*FAM175A*	*MET*	*PTEN*	*SPRED1*
BMPR1A	*FANCA*	*MITF*	*RAD50*	*STK11*
BRCA1	*FANCB*	*MLH1*	*RAD51*	*SUFU*
BRCA2	*FANCC*	*MRE11A*	*RAD51B*	*TERF2IP*
BRIP1	*FANCD2*	*MSH2*	*RAD51C*	*TERT*
CASR	*FANCE*	*MSH3*	*RAD51D*	*TMEM127*
CDC73	*FANCF*	*MSH6*	*RB1*	*TP53*
CDH1	*FANCG*	*MUTYH*	*RECQL4*	*TSC1*
CDK4	*FANCI*	*NBN*	*RET*	*TSC2*
CDKN1B	*FANCL*	*NF1*	*RHBDF2*	*VHL*
CDKN2A	*FANCM*	*NF2*	*RINT1*	*WT1*
CEBPA	*FH*	*NSD1*	*RUNX1*	*XPA*
CHEK2	*FLCN*	*NTHL1*	*SDHA*	*XPC*
CTRC	*GALNT12*	*PALB2*	*SDHAF2*	*XRCC2*
DDB2	*GATA2*	*PDGFRA*	*SDHB*	
DICER1	*GPC3*	*PHOX2B*	*SDHC*	

a. For the complete list of SNPs included in the panel, visit www.illumina.com/TruSightHereditaryCancer.

Table 2: Sample batching and output variation between instruments and reagent kits

Sequencing system[a]	Reagent Kit	Single reads	Output	Run time	Sample plexity[b]
iSeq 100 System	100 i1	4M	1.2 Gb	19 hr	2
MiSeq and MiSeqDx Systems	v2 Micro	4M	1.2 Gb	19 hr	2
	v2 Standard	15M	4.5 Gb	24 hr	9
	v3 Standard	25M	7.5 Gb	28 hr	16
MiniSeq System	Mid Output	8M	2.4 Gb	17 hr	5
	High Output	25M	7.5 Gb	24 hr	16
NextSeq 550 and NextSeq 550Dx Systems	Mid Output	130M	39 Gb	26 hr	80
	High Output	400M	120 Gb	39 hr	256

a. Theoretical outputs and times for the iSeq 100 and MiniSeq Systems are based on instrument specifications. Internal verification for the TruSight Hereditary Cancer Panel was performed on the MiSeq and NextSeq 550 Systems only.
b. Sample throughput is based on 300× average coverage per sample.

Table 4: Variant detection in Horizon Discovery samples using TruSight Hereditary Cancer Panel[a,b]

						Observed MAF at varied DNA inputs[c]		
Sample	Gene	Variant	Variant type	Consequence	Expected MAF	50 ng	25 ng	10 ng
HD793	*BRCA1*	P871L	SNV	missense mutation	100%	100%	100%	99.8%
	BRCA1	S1613G	SNV	missense mutation	50%	49.8%	47.7%	45.8%
	BRCA1	K1183R	SNV	missense mutation	50%	45.0%	43.9%	44.9%
	BRCA1	K820E	SNV	missense mutation	50%	48.1%	43.6%	45.6%
	BRCA1	D435Y	SNV	missense mutation	50%	42.8%	46.3%	44.6%
	BRCA2	V2466A	SNV	missense mutation	100%	99.9%	100%	100%
	BRCA2	N289H	SNV	missense mutation	50%	39.2%	40.5%	40.5%
	BRCA2	N991D	SNV	missense mutation	50%	48.6%	48.1%	48.0%
	BRCA2	N1784fs	Deletion	frameshift mutation	50%	42.2%	35.7%	38.9%
	BRIP1	S919P	SNV	missense mutation	100%	99.7%	99.9%	100%
	NBN	E185Q	SNV	missense mutation	50%	41.1%	35.1%	38.5%
HD794	*BARD1*	R378S	SNV	missense mutation	50%	50.5%	49.9%	48.0%
	BRCA2	V2466A	SNV	missense mutation	100%	99.9%	99.9%	99.8%
	BRCA2	I2675fs	Insertion	frameshift mutation	50%	41.0%	40.9%	40.3%
	BRIP1	S919P	SNV	missense mutation	100%	99.9%	100%	100%
	NBN	E185Q	SNV	missense mutation	100%	100%	100%	100%

a. Sequencing was performed on the MiSeq System.
b. Alignment and variant calling were performed with the DRAGEN Enrichment App. Observed minor allele.
c. frequency (MAF) values are mean values from four technical replicates.

Table 5: Variant detection in collaboration samples using TruSight Hereditary Cancer Panel[a,b]

Sample	Gene	Reference allele	Variant allele	Variant type	Consequence	Rep 1 MAF[c]	Rep 2 MAF[c]
1	*PALB2* overlap			CNV	copy number change	Detected	Detected
2	*RB1*	T	TTCAAAA	Insertion	In frame insertion	54.1%	53.6%
	TSC2	C	T	SNV	Stop gained	49.8%	47.5%
3	*POLE*	C	T	SNV	Missense variant	44.1%	47.0%
4	*CHEK2*	A	G	SNV	Missense variant	40.8%	44.9%
5	*MSH6*	GA	G	Deletion	Frameshift variant	50.9%	45.0%
6	*BRCA2*	CG	C	Deletion	Frameshift variant	29.9%	36.3%
7	*MLH1*	C	T	SNV	Stop gained	31.0%	31.9%
8	*BRCA1*	T	C	SNV	Missense variant	39.6%	35.1%

a. Sequencing was performed on the MiSeq System.
b. Alignment and variant calling with the DRAGEN Enrichment App.
c. Observed variant calls correlate with genotypes previously reported by our collaborator (data not shown).

Cancer type	Recommended genes for screening
Breast	*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, STK11, TP53*
Colon	*APC, AXIN2, BMPR1A, CHEK2 EPCAM, GREM1, MLH1 MSH2, MSH6, PMS2, MSH3, MUTYH, NTLH1, POLD1, POLE, PTEN, SMAD4, STK11, TP53*
Ovarian	*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NBN, NF1, PALB2, PTEN, STK11, TP53*
Gastric	*CDH1*
Other	*MEN1, NF2, RB1, RET, SDHAF2, SDHB, SDHC, SDHD, TSC1/2, VHL, TP53, WT1*

Figure 2: Genes included that have known associations with genetic predisposition to specific types of cancers.

Fast library preparation and enrichment workflow

The TruSight Hereditary Cancer Panel uses Illumina DNA Prep with Enrichment, enabling fast library preparation, with sequencing-ready libraries ready in 6.5 hours, including only 2 hours hands-on time. A key component of the Illumina DNA Prep with Enrichment solution is on-bead tagmentation, which uses bead-bound transposomes to mediate a uniform tagmentation reaction (Figure 3). This strategy eliminates the need for separate DNA fragmentation steps. For gDNA inputs between 10–50 ng, saturation-based DNA normalization also eliminates the need for individual library quantification and normalization steps before enrichment. Target enrichment occurs through proven hybrid–capture chemistry, enabling reliable detection of relevant variants for SNVs, indels, and CNVs. Libraries are hybridized to biotin-labeled probes specific for targeted DNA regions. Targets are captured by streptavidin magnetic beads that bind to the biotinylated probes, pulling the bound fragments from solution. After captured fragments are eluted from the beads, the targeted library is ready for sequencing.

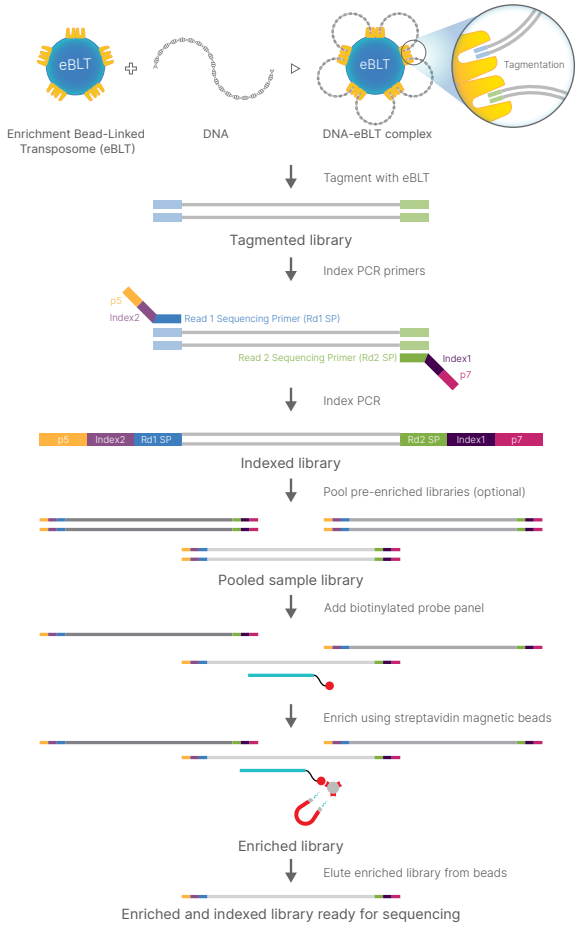

Figure 3: Illumina DNA Prep with Enrichment workflow—A uniform tagmentation reaction mediated by eBLTs followed by a single hybridization reaction enables a fast and flexible workflow.

Accurate data

With the ability to assess 113 genes per sample, the TruSight Hereditary Cancer Panel provides a high level of sample throughput while maintaining excellent specificity and uniformity. To demonstrate assay performance, sequencing metrics from two sequencing systems were analyzed using research collaborator samples. Eight samples (in duplicate) with 50 ng DNA input were prepared using Illumina DNA Prep with Enrichment with eight-plex enrichments and sequenced on the MiSeq System and the NextSeq System, and data were evaluated using the BaseSpace Enrichment App v3.1.0. Results showed a high percentage of coverage uniformity (Figure 4).

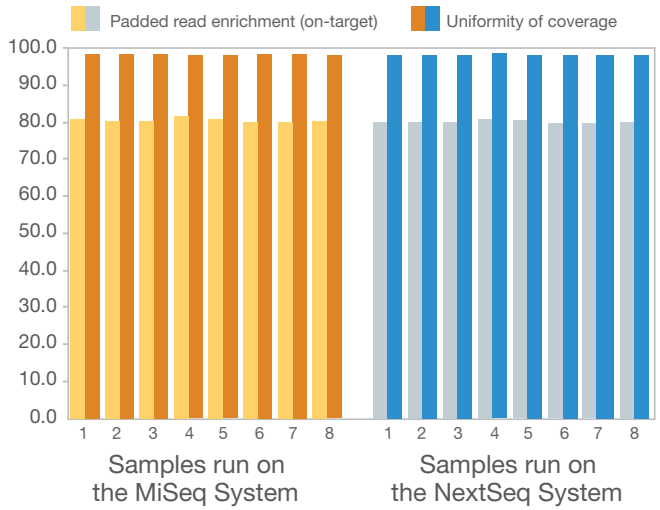

Figure 4: On-target alignment and coverage uniformity—DNA extracted from collaborator samples were prepared using the TruSight Hereditary Cancer Panel and sequenced on the (left) MiSeq System and (right) NextSeq 550 System. Mean values from two technical replicates are shown for each sample.

Variant calling

To demonstrate variant calling performance at different input levels, sets of 16 samples were prepared with 10 ng, 25 ng, and 50 ng DNA inputs. Sample sets were comprised of four replicates each of Horizon Discovery (HD) samples BRCA Germline I Reference Standard gDNA HD793 and BRCA Germline II Reference Standard gDNA HD794. Each input level was sequenced in 16-plex after preparing with Illumina DNA Prep with Enrichment with 8-plex enrichments. Sequencing was performed on the MiSeq System and data was evaluated using the DRAGEN Enrichment App. Results were concordant to the published list for Horizon Discovery for samples HD793 and HD794, demonstrating reproducible results across all input levels tested. Additional analysis was performed on samples containing unknown variants from research collaborators (Table 4). 50 ng DNA input of eight samples in duplicate were prepared using Illumina DNA Prep with Enrichment with eight-plex enrichments and sequenced on the MiSeq System. Using the DRAGEN Enrichment App for data analysis, variants from different classes (SNV, CNV, and indel) were detected (Table 5), which correlated with genotypes previously reported by our collaborator.

DRAGEN Enrichment App or the BaseSpace Enrichment App can be used for variant calling to provide results in VCF format. Customers can select any third-party tertiary analysis platform to annotate and interpret variants.

For more information, including adjustable assay parameters for the Illumina DNA Prep with Enrichment workflow and the impact on Variant Calling, read the User-definable parameters in the Illumina DNA Prep with Enrichment workflow tech note and the Analyze germline CNVs with TruSight Hereditary Cancer Panel tech note.

Summary

The TruSight Hereditary Cancer Panel enables researchers to access an expert-defined content set for analyzing variation within genes previously linked with a predisposition towards cancer. The optimized probe set provides comprehensive coverage of the targeted regions with high coverage uniformity for identifying many variants. Combining this content with the Illumina DNA Prep with Enrichment method enables a fast, easy workflow with a low sample input requirement, and the flexibility of using any Illumina benchtop sequencing system. The TruSight Hereditary Cancer Panel is a highly efficient targeted sequencing solution to accelerate detection of variants associated with cancer predisposition.

Learn more

TruSight Hereditary Cancer Panel

References

1. Illumina. Illumina DNA Prep with Enrichment data sheet. Published 2020. Accessed September 5, 2023.
2. Mavaddat N, Pharoah PD, Michailidou K, et al. Prediction of breast cancer risk based on profiling with common genetic variants. *J Natl Cancer Inst*. 2015;107(5):djv036. Published 2015 Apr 8. doi:10.1093/jnci/djv036
3. University of Cambridge, Centre for Cancer Genetic Epidemiology. Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) ccge.medschl.cam.ac.uk/boadicea/. Accessed September 5, 2023.

Ordering information

Product	Catalog no.
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 Prep with Enrichment, (S) Tagmentation (96 Samples)	20025524
Illumina DNA Prep with Enrichment, (S) Tagmentation (16 Samples)	20025523
Illumina DNA Prep, (S) Tagmentation (96 Samples)	20025520
Illumina DNA Prep, (S) Tagmentation (16 Samples)	20025519
iSeq 100 i1 Reagent	20021533
iSeq 100 i1 Reagent	20021534
MiSeq Reagent Micro Kit v2	MS-103-1002
MiSeq Reagent Kit v2	MS-102-2002
MiSeq Reagent Kit v3	MS-102-3003
MiniSeq Mid Output Kit	FC-420-1004
MiniSeq High Output Kit	FC-420-1003
NextSeq 500/550 Mid Output Kit v2.5	20024905
NextSeq 500/550 High Output Kit v2.5	20024908
Flex Lysis Reagent Kit	20018706

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