illumina®

TruSight™ Hereditary Cancer Panel

Expert-defined content targeting genes associated with a predisposition for various cancers delivered on proven next-generation sequencing technology.

Highlights

- **Comprehensive content for assessing germline mutations**
  Panel includes 113 expert-selected genes associated with hereditary cancer predisposition
- **Fast workflow with Nextera™ Flex for Enrichment**
  Easy protocol enables library preparation and enrichment in 6.5 hours, with 2 hours hands-on time
- **Flexible options with Illumina systems**
  Compatibility with all Illumina benchtop sequencers enables sample throughput ranging from 2–256 samples per run
- **High-quality sequence data**
  Hybrid-capture enrichment enables good coverage uniformity for accurate detection of SNVs, indels, and CNVs

Introduction

As we learn more about the role genetic variants play in cancer predisposition, researchers will benefit from the ability to perform comprehensive evaluation of the genes in which these variants lie. The TruSight Hereditary Cancer Panel provides labs with this ability. 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 identification purposes 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. The assay uses hybrid-capture chemistry integrated with Nextera Flex for Enrichment, the newest library prep chemistry from Illumina.[1] Nextera Flex for Enrichment is 85% faster than standard Illumina library prep and enrichment, using an innovative bead-based chemistry with a simplified, single hybridization step. Nextera Flex for Enrichment is also compatible with all Illumina benchtop sequencers, offering flexibility in experimental design with a wide range of sample throughput (Table 1). Combining the speed of Nextera with the MiSeq™ System, the entire workflow (Figure 1) can be completed in 48 hours from sample to data.

Table 1: TruSight Hereditary Cancer Panel specifications

Parameter	Details
System	iSeq™ 100 System, MiniSeq™ System, MiSeq System, 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 hours total time, 2 hours 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 (accessory product).

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 (Figure 2, Table 2).

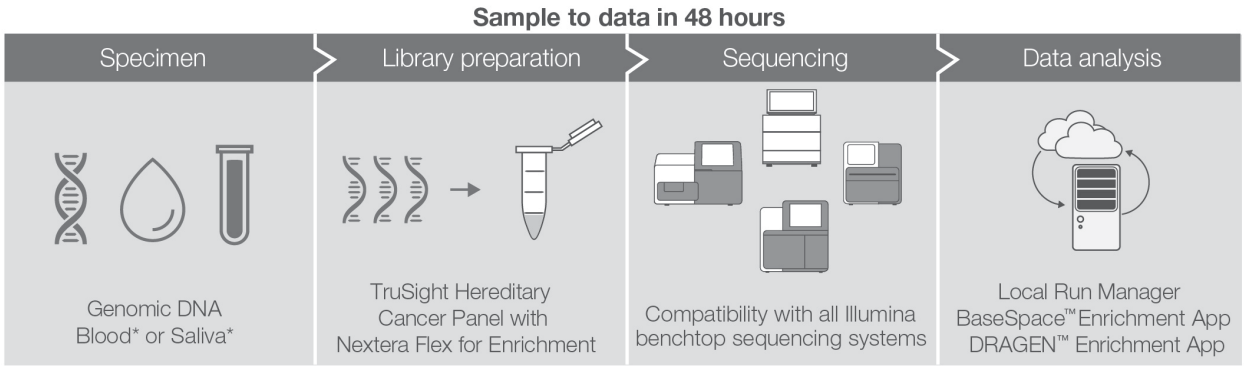

Figure 1: Fast, flexible NGS workflow— The TruSight Hereditary Cancer Panel was developed with the Nextera Flex library prep chemistry, which integrates library preparation and enrichment steps. A fast, streamlined, and optimized workflow delivers fully enriched libraries in just 6.5 hours. TruSight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

*Extraction directly from blood or saliva requires use of the Flex Lysis Reagent Kit (accessory product).

Figure 2: Range of throughput available with TruSight Heredity Cancer Panel on four Illumina sequencing systems

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

Sequencing System[a]	Kit	Single reads	Output	Runtime	Sample plexity[b]
iSeq 100 System	100 i1	4 M	1.2 Gb	19 hours	2
MiSeq Series	v2 Micro	4 M	1.2 Gb	19 hours	2
	v2 Standard	15 M	4.5 Gb	24 hours	9
	v3 Standard	25 M	7.5 Gb	28 hours	16
MiniSeq System	Mid Output	8 M	2.4 Gb	17 hours	5
	High Output	25 M	7.5 Gb	24 hours	16
NextSeq Series	Mid Output	130 M	39 Gb	26 hours	80
	High Output	400 M	120 Gb	39 hours	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 Systems only.

b. Sample throughput is based on 300× average coverage per sample.

Comprehensive content design

The TruSight Hereditary Cancer Panel includes an extensive list of genes commonly associated with hereditary predisposition to breast, colon, ovarian, colon, and gastric cancers. The content was developed with input and feedback from key opinion leaders on genetic risk assessment. The panel includes 10,341 probes that target 113 genes (Table 3) related to cancer predisposition, recommended in key guidelines (Figure 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) from a single assay.

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 3: Genes included in key guidelines associated with risk reduction

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*	

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

Fast library preparation and enrichment workflow

The TruSight Hereditary Cancer Panel uses Nextera Flex for Enrichment, which enables the library prep workflow to be completed in 6.5 hours with only 2 hours hands-on time. A key component of the Nextera Flex for Enrichment solution is On-Bead Tagmentation, which uses bead-bound transposomes to mediate a uniform tagmentation reaction (Figure 4). 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 adding streptavidin magnetic beads that bind to the biotinylated probes, then pulling the bound fragments from solution. After captured fragments are eluted from the beads, the targeted library is ready for sequencing.

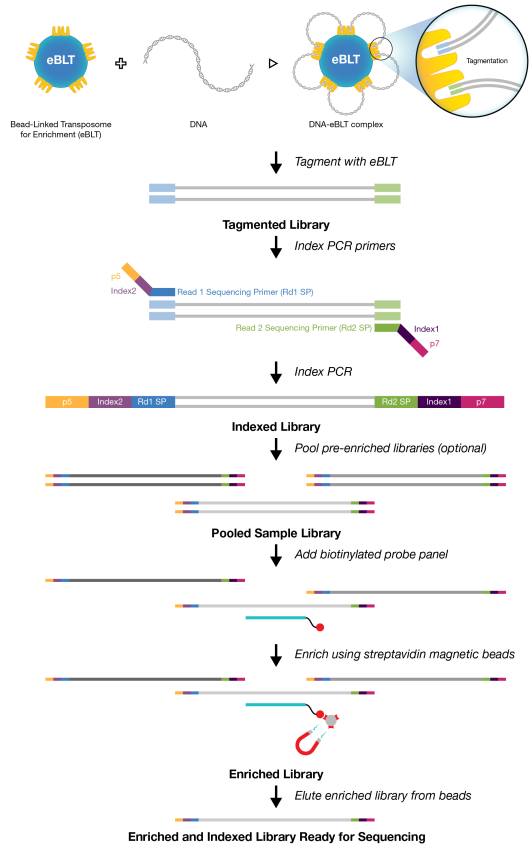

Figure 4: Nextera Flex for Enrichment workflow—A uniform tagmentation reaction mediated by eBLTs followed by a single hybridization reaction enables a fast and flexible workflow.

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. 50 ng DNA input of eight samples in duplicate were prepared using Nextera Flex for Enrichment with 8-plex enrichments and sequenced on the MiSeq System and the NextSeq System, and data was evaluated using the BaseSpace Enrichment App version 3.1.0. Results showed high percentage of coverage uniformity s (Figure 5).

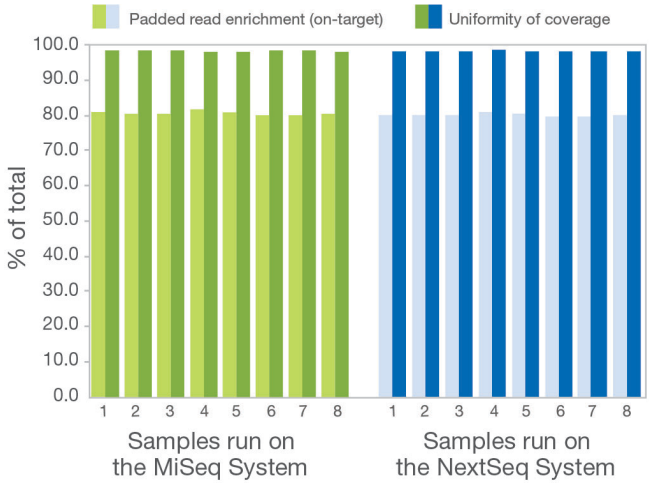

Figure 5: On-target alignment and coverage uniformity—DNA extracted from collaborator samples were prepared using the TruSight Hereditary Cancer Panel and sequenced on the MiSeq System (left) and the NextSeq System (right). Mean values from two technical replicates are shown for each sample.

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 Nextera Flex for 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 (Table 4).

Additional analysis was performed on samples containing unknown variants from research collaborators. 50 ng DNA input of eight samples in duplicate were prepared using Nextera Flex for Enrichment with 8-plex enrichments and sequenced on the MiSeq System. Using the DRAGEN Enrichment App for data analysis, variants from different classes (SNV, indel, and CNV) were detected (Table 5), which

Table 4: Variant detection in Horizon Discovery samples with TruSight Hereditary Cancer Panel and the DRAGEN Enrichment App

						Observed MAF at varied DNA inputs		
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%

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

Table 5: Detection of variants in collaboration samples with TruSight Hereditary Cancer Panel and the DRAGEN Enrichment App

Sample	Gene	Reference allele	Variant Allele	Variant Type	Consequence	Rep 1 MAF	Rep 2 MAF
1	*PALB2* overlap			CNV	copy number change (loss of exons 7–13)	Detected	Detected
2	*RB1*	T	TTCAAAA	Insertion	Inframe 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%

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

correlated with genotypes previously reported by our collaborator. The 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.

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 Nextera Flex for 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

For more information about the TruSight Hereditary Cancer Panel, visit www.illumina.com/TruSightHereditaryCancer

References

1. Illumina (2018). Nextera Flex for Enrichment data sheet.
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). pii:djv036. doi: 10.1093/jnci/djv036.
3. BOADICEA-Centre for Cancer Genetic Epidemiology. (ccge.medschl.cam.ac.uk/boadicea/). Accessed November 17, 2019.

Ordering information

Product	Category	Catalog no.
TruSight Hereditary Cancer - Enrichment Oligos Only	Enrichment oligos (8 enrichment reactions, up to 12 samples per enrichment)	20029551
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Library prep and enrichment reagents (96 samples, 8 x 12-plex enrichment reactions)	20025524
Nextera DNA Flex Pre-Enrichment Library Prep and Enrichment Reagents	Library prep and enrichment reagents (16 samples, 16 x 1-plex enrichment reactions)	20025523
Nextera DNA Flex Pre-Enrichment Library Prep Reagents	Library prep and enrichment reagents (96 samples)	20025520
Nextera DNA Flex Pre-Enrichment Library Prep Reagents	Library prep and enrichment reagents (16 samples)	20025519
IDT for Illumina Nextera DNA Unique Dual Indexes Set A	Index adapters (96 Indexes, 96 Samples)	20027213
IDT for Illumina Nextera DNA Unique Dual Indexes Set B	Index adapters (96 Indexes, 96 Samples)	20027214
IDT for Illumina Nextera DNA Unique Dual Indexes Set C	Index adapters (96 Indexes, 96 Samples)	20027215
IDT for Illumina Nextera DNA Unique Dual Indexes Set D	Index Adapters (96 Indexes, 96 Samples)	20027216
iSeq 100 i1 Reagent	300-cycle single kit	20021533
iSeq 100 i1 Reagent 4 Pack	300-cycle quad kit	20021534
MiSeq Reagent Micro Kit v2	300 cycle kit	MS-103-1002
MiSeq Reagent Kit v2	300 cycle kit	MS-102-2002
MiSeq Reagent Kit v3	600 cycle kit	MS-102-3003
MiniSeq Mid Output Kit	300 cycle kit	FC-420-1004
MiniSeq High Output Kit	300 cycle kit	FC-420-1003
NextSeq 500/550 Mid Output Kit v2.5	300 cycle kit	20024905
NextSeq 500/550 High Output Kit v2.5	300 cycle kit	20024908
Flex Lysis Reagent Kit	96 reactions	20018706

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

illumina®

For Research Use Only. Not for use in diagnostic procedures.