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TruSight™ Cancer Sequencing Panel

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

Highlights

- **Expert-Defined Content**
  Chosen by The Institute of Cancer Research, London to target genes associated with a predisposition towards cancer
- **Low Input DNA Requirement**
  Excellent data quality with as little as 50 ng DNA to preserve precious samples
- **Fast, Simple Workflow**
  Sample preparation and enrichment completed in 1.5 days

Introduction

As we learn more about the role genetic variants play in cancer predisposition, researchers will need to be able to comprehensively evaluate the genes in which these variants lie. This process is now simpler and faster with the TruSight Cancer Sequencing Panel. Developed in collaboration with experts in cancer genomics, the pre-designed, ready-to-use oligos specifically target genes associated with cancer. The sequencing panel is compatible with TruSight Rapid Capture kits, providing a single, integrated sample preparation and enrichment workflow that can be completed in just 1.5 days (Figure 1). Delivering excellent data quality from low sample input (50 ng), TruSight Cancer and TruSight Rapid Capture enable researchers to analyze precious samples, while retaining sufficient material for future analyses.

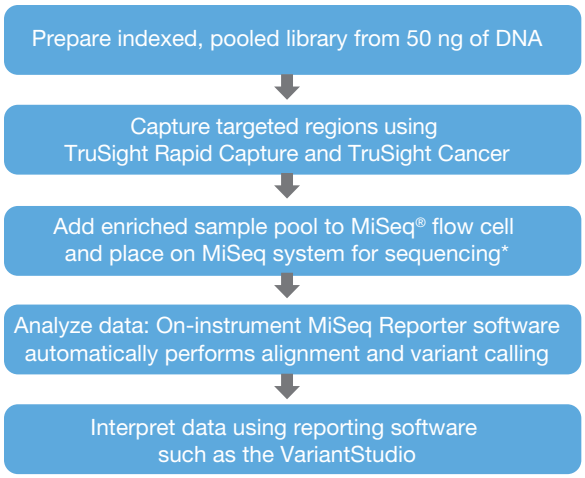
Content Design Strategy

Developed in collaboration with Professor Nazneen Rahman and team at The Institute of Cancer Research (ICR), London, the TruSight Cancer Sequencing Panel targets 94 genes suspected to play a role in predisposing to cancer, including genes associated with both common (e.g., breast, colorectal) and rare cancers. In addition, the panel includes 284 SNPs suspected to be associated with cancer through genome-wide association studies (GWAS). Content selection was based on expert curation of the scientific literature and other high-quality resources.

Superior Coverage

TruSight Cancer features a highly optimized probe set that delivers comprehensive coverage of genes suspected to indicate a predisposition for cancer, starting from only 50 ng of DNA input. The kit includes ~4,000 80-mer probes, each constructed against the human 170–220 NCBI37/hg19 reference genome. The probe set was designed to enrich for > 1,700 exons, spanning 94 genes of interest (Table 1).

Figure 1: Integrated Workflow



TruSight Cancer is compatible with the TruSight Rapid Capture method, which integrates library sample preparation and enrichment steps to offer a fast, streamlined, and optimized workflow, delivering fully enriched libraries for up to 96 samples in just 1.5 days.

* For higher throughout, use HiSeq® flow cells and the HiSeq system.

The TruSight Cancer Sequencing Panel targets a total of 255 Kb of the human genome. The 80-mer probes target libraries of approximately 500 bp (insert size of 300 bp), enriching 350–650 bases centered symmetrically around the midpoint of the probe (Figure 2)[1]. This means that the kit provides coverage of exonic and non-coding DNA in exon-flanking regions, on average 50 bp.

Integrated Library Preparation and Enrichment Workflow

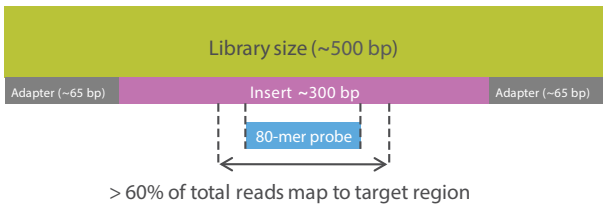
TruSight Cancer and TruSight Rapid Capture leverage the speed of Nextera® library preparation technology. By eliminating the need for mechanical DNA fragmentation and introducing a unique multiplex pre-enrichment sample pooling, the TruSight Rapid Capture method reduces hands-on time for a high-throughput workflow that saves at least one full day over all other currently available enrichment workflows (Figure 1). Furthermore, master-mixed reagents are coupled with a plate-based protocol for simultaneous processing of up to 24 enrichment reactions (288 total samples).

Flexible kit configurations enable labs to readily meet their sample throughput needs. For those requiring higher throughput, kit reagent volumes are optimized for liquid handlers to make an automation-

Table 1: Coverage Details

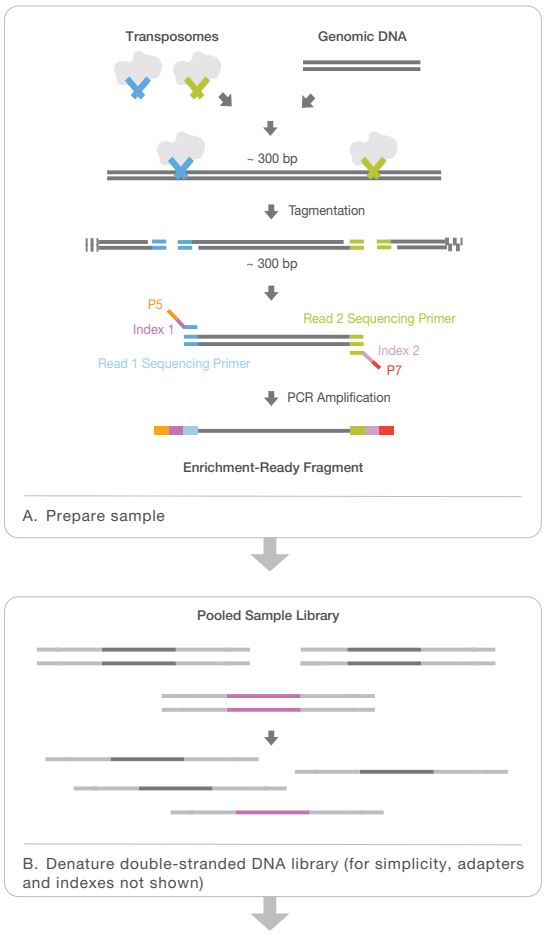
Cumulative target region size	255 Kb
Number of target genes	94
Number of target exons	> 1,700
Probe size	80-mer
Number of probes	~4,000
Recommended mean coverage	100×
Target minimum coverage	20×
Percent exons covered based on coverage metrics	≥ 95%

Figure 2: Probe Footprint



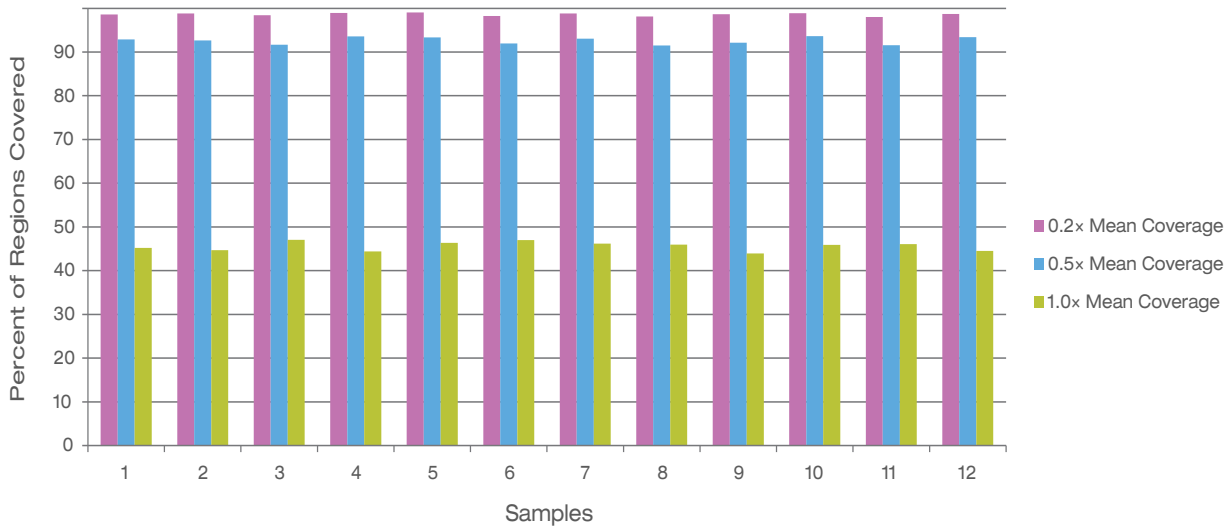
With an approximately 500 bp DNA library (insert size of 300 bp), the probe will enrich 250–650 bp centered around its midpoint.

Figure 3: Integrated TruSight Rapid Capture Workflow



The TruSight Rapid Capture workflow provides a fast, simple method for isolating the genes targeted using TruSight Cancer. The streamlined, automation-friendly workflow combines library preparation and enrichment steps, and can be easily completed in 1.5 days with minimum hands-on time.

Figure 4: High Coverage Uniformity



Coverage uniformity is given for 12 samples with respect to the percentage of targeted regions at varying mean normalized read depths. The 12 samples were prepared and then simultaneously enriched using the TruSight Rapid Capture along with the TruSight Cancer Sequencing Panel. Pooled samples were sequenced across a MiSeq® standard flow cell, generating mean read depths of 130–230× (varying for each sample). Over 95% of bases (~250 Kb) were covered at 0.2× mean coverage.

friendly workflow. TruSight Rapid Capture kits supporting lower throughput options are also available, allowing labs to cost-effectively run samples immediately instead of waiting to batch.

The process starts with rapid Nextera-based sample prep to convert input genomic DNA into adapter-tagged libraries (Figure 3A). This rapid prep requires only 50 ng of input DNA and takes less than 3 hours for a plate of 96 samples. Nextera tagmentation of DNA simultaneously fragments and tags DNA without the need for mechanical shearing. Integrated sample barcodes then allow the pooling of up to 96 samples for a single Rapid Capture pull down. Next, libraries are denatured into single-stranded DNA (Figure 3B) and biotin-labeled probes specific to the targeted region are used for the Rapid Capture hybridization (Figure 3C). The pool is enriched for the desired regions by adding streptavidin beads that bind to the biotinylated probes (Figure 3D). Biotinylated DNA fragments bound to the streptavidin beads are magnetically pulled down from the solution (Figure 3E). The enriched DNA fragments are then eluted from the beads and hybridized for a second Rapid Capture. This entire process is completed in only 1.5 days, enabling a single researcher to efficiently process up to 288 samples at one time—all without automation.

Data Analysis

Sequence data generated from TruSight Cancer enriched libraries are analyzed by the on-instrument MiSeq Reporter (MSR) software. After demultiplexing and FASTQ file generation, the software uses the Burrows-Wheeler Aligner (BWA) to align the reads against the hg19 homo sapiens reference genome to create BAM files. The Genome Analysis Toolkit (GATK) is then used to perform variant analysis for the target regions specified in the manifest file. The output of GATK are VCF files, which are text files that contain SNPs, indels, and other structural variants.

High Data Quality

With TruSight Cancer and TruSight Rapid Capture, researchers can be confident in the quality of sequencing data generated from pooled multisample libraries. Each sample is sequenced with high coverage uniformity across the target region, with 95% of exons covered at a minimum coverage of 20× (Figure 4). This uniformity applies to smaller exons (< 150 bp) as well as long coding exons.

Summary

The TruSight Cancer Sequencing 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 a large number of variants. Combining this content with the TruSight Rapid Capture method enables a fast, easy workflow, requiring low sample DNA input, generating a highly efficient resequencing solution to accelerate detection of genes associated with cancer.

Learn More

To learn more about TruSight Cancer Sequencing Panel, TruSight Rapid Capture kits, and Illumina next-generation sequencing technology, visit www.illumina.com/trusightcancer.

Reference

1. Optimizing Coverage for Targeted Resequencing Technical Note (www.illumina.com/documents/products/technotes/technote_optimizing_coverage_for_targeted_resequencing.pdf)

Ordering Information

Product	Catalog No.	TG Catalog No.*
TruSight Cancer Sequencing Panel (4 enrichments)	FC-121-0202	TG-141-1002
Rapid Capture Kits		
TruSight Rapid Capture (1 Index, 8 Samples)	FC-140-1101	TG-140-1101
TruSight Rapid Capture (2 Indices, 8 Samples)	FC-140-1102	TG-140-1102
TruSight Rapid Capture (4 Indices, 16 Samples)	FC-140-1103	TG-140-1103
TruSight Rapid Capture (24 Indices, 48 Samples)	FC-140-1104	TG-140-1104
TruSight Rapid Capture (24 Indices, 96 Samples)	FC-140-1105	TG-140-1105
TruSight Rapid Capture (96 Indices, 288 Samples)	FC-140-1106	TG-140-1106

* TG-labeled consumables include features intended to help customers reduce the frequency of revalidation. They are available only under supply agreement and require customers to provide a binding forecast. TruSight sequencing panels are available for evaluation purposes prior to executing a supply agreement. Please contact your account manager for more information.

Note regarding biomarker patents and other patents unique to specific uses of products.

Some genomic variants, including some nucleic acid sequences, and their use in specific applications may be protected by patents. Customers are advised to determine whether they are required to obtain licenses from the party that owns or controls such patents in order to use the product in customer's specific application.

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