TruSight™ One Sequencing Panel

The largest sequencing panel available, targeting > 4,800 genes and enabling labs to expand and streamline their sequencing portfolio.

Highlights

- **Unmatched Content and Performance**
  Targets > 4,800 genes associated with human diseases
- **Single Panel Replaces Many**
  Expand and streamline your sequencing portfolio with one assay and one workflow
- **Flexible Reporting**
  VariantStudio software enables push-button gene filtering for easy exploration of genomic variant data

Introduction

The TruSight One Sequencing Panel provides comprehensive coverage of > 4,800 clinically relevant genes. Laboratories can analyze all of the genes on the panel or choose to focus on a specific subset. In this way, a single panel can effectively replace all other sequencing panels. For those needing bioinformatics capabilities, TruSight One comes with VariantStudio software, a simple, yet powerful tool for analysis, classification, and reporting of genomic variants. Now there's just one workflow to follow and one procedure for managing genomic samples. The result is an integrated sample-to-report process capable of supporting an entire next-generation sequencing (NGS) portfolio (Figure 1).

Comprehensive Assay

The TruSight One Sequencing Panel covers 12 Mb of genomic content, including 4,813 genes associated to a clinical phenotype. This enables labs to focus on genes with proven relevance, rather than wading through excess data that may not be of immediate value. The panel is designed for trio sequencing on a MiSeq® or 36 samples on a HiSeq® 2500/1500 Rapid Run, while achieving 20× minimum depth of coverage at more than 95% of the targets*.

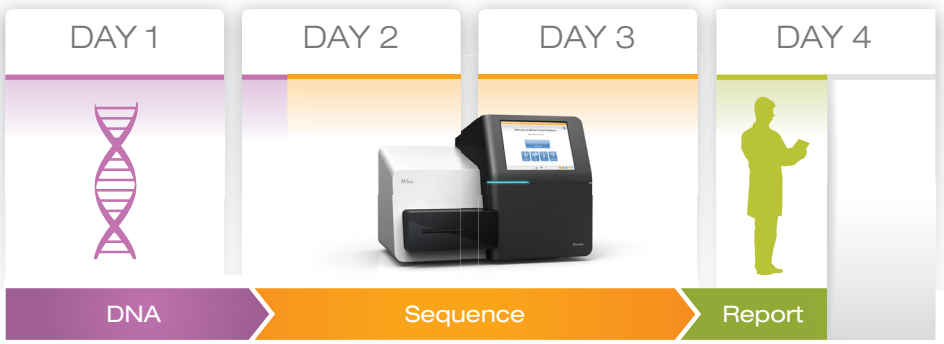
Content Design Strategy

Developed by Illumina, the TruSight One Sequencing Panel focuses on the exonic regions harboring disease-causing mutations. It was specifically designed to cover the most commonly ordered molecular assays. Using TruSight One, labs can now perform all of these investigations on-site, with one panel. Genomic targets were identified based on information in the Human Gene Mutation Database (HGMD)[1], the Online Mendelian Inheritance in Man (OMIM) catalog[2], GeneTests.org[3], Illumina TruSight sequencing panels[4], and other commercially available sequencing panels. Combining data from these sources ensures that the TruSight One panel covers all genes currently reviewed in clinical research settings for a truly comprehensive assay.

Superior Coverage

The TruSight One Sequencing Panel features a highly optimized probe set that supports analysis of a large number of variants. Starting with only 50 ng of high-quality DNA input, the panel delivers comprehensive coverage of the targeted exonic sequences. The panel includes > 125,395 80-mer probes, each constructed against the human NCBI37/hg19 reference genome. The probe set was designed to enrich for ~62,000 exons, spanning 4,813 genes of interest (Table 1). TruSight One probes were made using an iterative process of design and functional testing to ensure the highest performance and uniformity, optimizing sequencing capacity and enabling unmatched multiplexing. The result is at least 20× coverage of 95% of the regions on the panel.

Figure 1: One Seamless Sample-to-Report Workflow



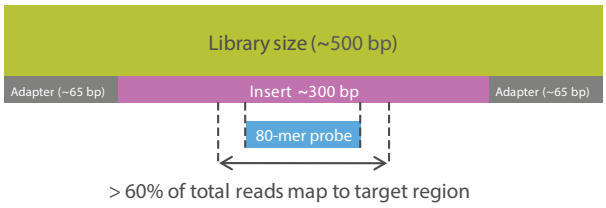
The Illumina TruSight One Sequencing Panel and VariantStudio software allow you to go from DNA sample to report in just four days.*

* Average time for a targeted gene panel. Times may vary depending on panel used.

Table 1: Coverage Details

Cumulative target region size	12 Mb
Number of target genes	4,813
Number of target exons	~62,000
Probe size	80-mer
Number of probes	125,395
Target minimum coverage	20×

Figure 2: Probe Footprint



With an approximately 500 bp DNA library (insert size of 300 bp), the probe will enrich 350–650 bp centered around its midpoint.

TruSight One targets 12 Mb 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)[5]. This means that, in addition to comprehensive coverage of the major exon regions, the panel provides coverage of exon-flanking regions (splice sites). Focusing on the subset of the exome with known associations to inherited disease (as indicated by HGMD, OMIM, GeneTests.org, and more) enables labs to detect variants that affect gene function more efficiently than whole-genome or whole-exome sequencing[6].

Flexible, Customizable Data Enrichment

VariantStudio software tool enables researchers to explore variant data easily, identify biologically relevant variants quickly, and enrich data with biological context. When used with the TruSight One Sequencing Panel, labs can quickly and accurately extract and report on only the disease-relevant information of interest. Using the intuitive software interface, users can effectively create subpanels from a single TruSight One analysis.

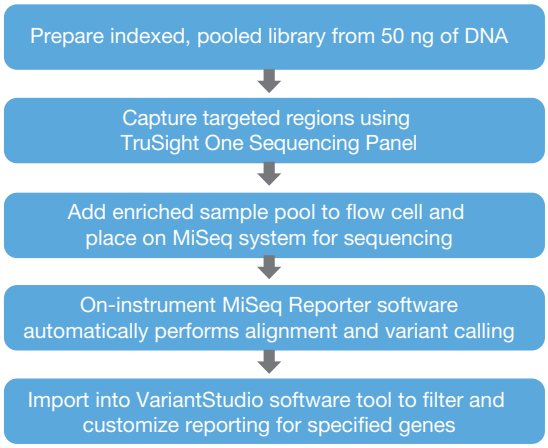
How it Works

TruSight One leverages the speed of Nextera® library preparation technology for a single, integrated sample preparation and enrichment workflow that can be completed in just 1.5 days (Figure 3).

One Workflow

TruSight One uses a unique multiplex pre-enrichment sample pooling strategy that eliminates the need for mechanical DNA fragmentation and reduces the number of enrichments needed for a successful library preparation. This method reduces hands-on time for a high-throughput workflow that saves at least one full day over all other currently available enrichment workflows. Furthermore, master-mixed reagents are coupled with a plate-based protocol for simultaneous processing of multiple enrichment reactions.

Figure 3: Simple, Integrated Workflow



The TruSight One sample prep process starts with Nextera tagmentation which converts input genomic DNA into adpater-tagged libraries without the need for mechanical shearing (Figure 4A). This method requires only 50 ng of input DNA and takes less than 3 hours for a plate of 3 to 12 samples. Integrated sample barcodes allow the pooling of 3 to 12 samples for a single pulldown. Next, libraries are denatured (Figure 4B) and biotin-labeled probes specific to the targeted region are used for hybridization (Figure 4C). The pool is enriched for the desired regions by adding streptavidin beads that bind to the biotinylated probes (Figure 4D). Biotinylated DNA fragments bound to the streptavidin-coated beads are magnetically pulled down from the solution (Figure 4E). The enriched DNA fragments are then eluted from the beads and hybridized for a second capture. This entire process is completed in only 1.5 days, enabling a single technician to process up to 36 samples at one time—all without automation.

Perform the Sequencing

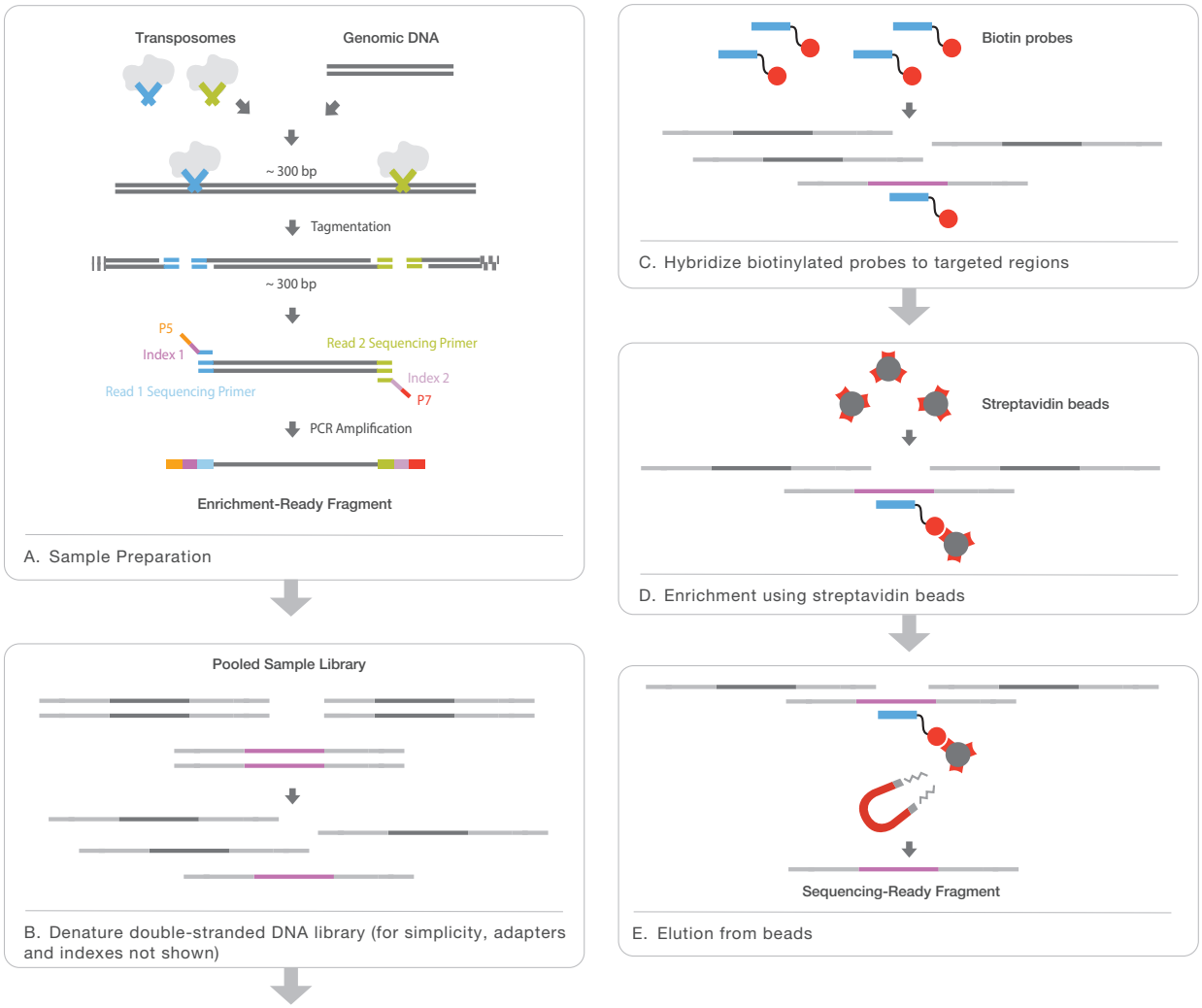
Prepared libraries are loaded on to a flow cell for sequencing with the MiSeq or HiSeq system. Simply place the flow cell into the instrument and run. Sequence data is exported as a .vcf file and imported easily in to the VariantStudio software for analysis.

Filtered Data Analysis

The TruSight One panel can be filtered to isolate a set of genes or regions for analysis and reporting, enabling one assay to represent multiple assays. Simply generate a gene list and select this list when importing .vcf data files from the MiSeq or HiSeq system into VariantStudio. For ease of use, VariantStudio software offers commonly applied filters, including variant quality, population frequency, functional impact, and known disease association.

In addition to single-sample filtering, VariantStudio software enables multisample comparisons that accelerate identification of causative variants. To support trio and pedigree filtering, the software provides

Figure 4: Seamless TruSight Rapid Capture Workflow



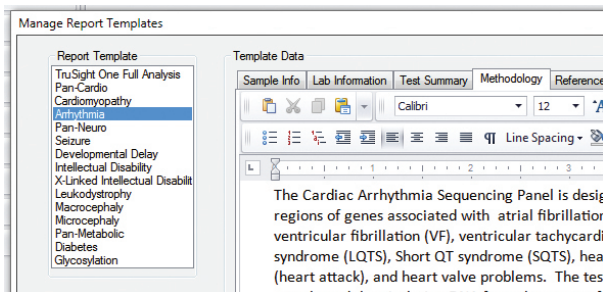
The TruSight One Sequencing Panel leverages Nextera library preparation technology to provide a fast, simple method for isolating targeted genes. The streamlined workflow combines library preparation and exome enrichment steps, and can be easily completed in 1.5 days with minimum hands-on time.

a collection of filters that identifies variants consistent with specified inheritance modes and patterns of disease progression.

Customizable Reporting

VariantStudio software enables users to customize reports to meet requirements specific to different diseases of interest and sequencing panels. Multiple report templates can be created and stored for later use. When a template is applied to a given sample, the user simply enters or imports sample-specific information from LIMS, combines it with the methodology, a summary of results, and the reported variant categories in VariantStudio (Figure 5). Reports, which are linked to the imported sample information, are then exported in PDF or rich-text formats for downstream use.

Figure 5: Templates Enable Customizable Reporting



VariantStudio provides guided template generation. Addition of sample-specific information enables customized reporting for different clinical research areas.

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Accurate Data

Whether sequencing on the MiSeq or HiSeq sequencing systems, the TruSight One Sequencing Panel yields unmatched uniformity and depth of coverage (Table 2). Trio sequencing on a single MiSeq instrument typically achieves 20× minimum depth of coverage at more than 95% of the targets.

Summary

The TruSight One Sequencing Panel and included VariantStudio data analysis software enable a seamless workflow. Using this comprehensive panel, labs can quickly sequence over 4,800 genes with an associated clinical phenotype. Filtering results with VariantStudio then enables creation of subpanels that are responsive to customer demands and advances in scientific understanding.

Learn More

To learn more about the TruSight One Sequencing Panel, visit www.illumina.com/trusightone.

References

1. Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php)
2. Online Mendelian Inheritance in Man (omim.org)
3. GeneTests (www.genetests.org)
4. Illumina TruSight Products (www.illumina.com/trusight)
5. Optimizing Coverage for Targeted Resequencing Technical Note.
6. www.illumina.com/documents/products/technotes/technote_optimizing_coverage_for_targeted_resequencing.pdf
7. Bainbridge MN, Wang M, Wu YQ, Newsham I, Muzny DM, et al. (2011) Targeted enrichment beyond the consensus coding DNA sequence exome reveals higher variant densities. Genome Biol. 12: R68.

Table 2: TruSight One Sequencing Panel Performance†

Sequencing Kit	Reads Passing Filter (M)	Read Length	Output (Gb)	Samples per Run	% Targets Covered at 12× Minimum	% Targets Covered at 20× Minimum
MiSeq Reagent Kit v3	22–25	2 × 150	7.5	3	97	95
HiSeq Rapid Run Mode (single flow cell)	up to 300	2 × 150	90	36	97	95
HiSeq Rapid Run Mode (dual flow cell)	up to 600	2 × 150	180	72	97	95

† Performance is reported for samples with > 50% enrichment. For the MiSeq system, this targets 1,200–1,400 K/mm^2 raw read density.

Ordering Information

Product	Catalog No.	TG Catalog No.*
TruSight One Sequencing Panel (9 samples)	FC-141-1006	TG-141-1006
TruSight One Sequencing Panel (36 samples)	FC-141-1007	TG-141-1007

* 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. Contact your account manager for more information.

*** Percentage is calculated by averaging the mean coverage for each exon, not each base.**

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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