illumina®

TruSight™ One Series of Sequencing Panels

Comprehensive panels curated to target specific, disease-associated regions of the exome with high analytical sensitivity and specificity.

Highlights

- **Extensive Content and High Coverage**
  Target up to 6700 genes associated with human disease with a minimum of 20× coverage with two panel options
- **Single Panel Replaces Iterative Testing**
  Consolidate and simplify your sequencing portfolio with one assay and one workflow
- **User-Defined, Flexible Reporting**
  Interrogate genomic variant data with user-defined gene filtering and report generation using VariantStudio or BaseSpace™ Variant Interpreter

Introduction

The TruSight One Sequencing Panels focus on exonic regions harboring known disease-causing mutations. Focusing on the subset of genes with known associations to inherited disease within the exome enables more efficient variant detection compared to whole-genome or whole-exome sequencing.[1] By combining data from multiple genomic databases and reviewing guidance from industry experts around the world, the TruSight One Series delivers a comprehensive set of disease-associated target regions designed to cover the most commonly ordered disease gene panels. With the TruSight One and TruSight One Expanded Panels, researchers can streamline and accelerate their sequencing studies by performing the same tests consolidated into a single assay.

The TruSight One Series

The TruSight One Sequencing Panel

Genomic targets with disease associations were identified in the Human Gene Mutation Database (HGMD),[2] the Online Mendelian Inheritance in Man (OMIM) catalog,[3] GeneTests.org,[4] previously developed Illumina TruSight sequencing panels,[5] and from direct input by industry experts (Figure 1). The TruSight One Sequencing Panel covers 12 Mb of genomic content, including > 4800 genes associated with specific clinical phenotypes. This enables researchers to focus their time and resources on genes with known disease associations.

The TruSight One Expanded Sequencing Panel

The TruSight One Expanded Sequencing Panel was developed under the same guiding principles as the original panel with further optimization to improve coverage in regions known to show suboptimal performance. The Expanded design targets 16.5 Mb of content including ~1900 additional genes with new disease associations in the reference databases. Although the NextSeq™ and HiSeq™ Series Systems are recommended for the TruSight One Expanded Panel (due to the larger content size), the TruSight One Panels are compatible with all Illumina Sequencing Systems and generate a minimum of 20× coverage on more than 95% of targets.*

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

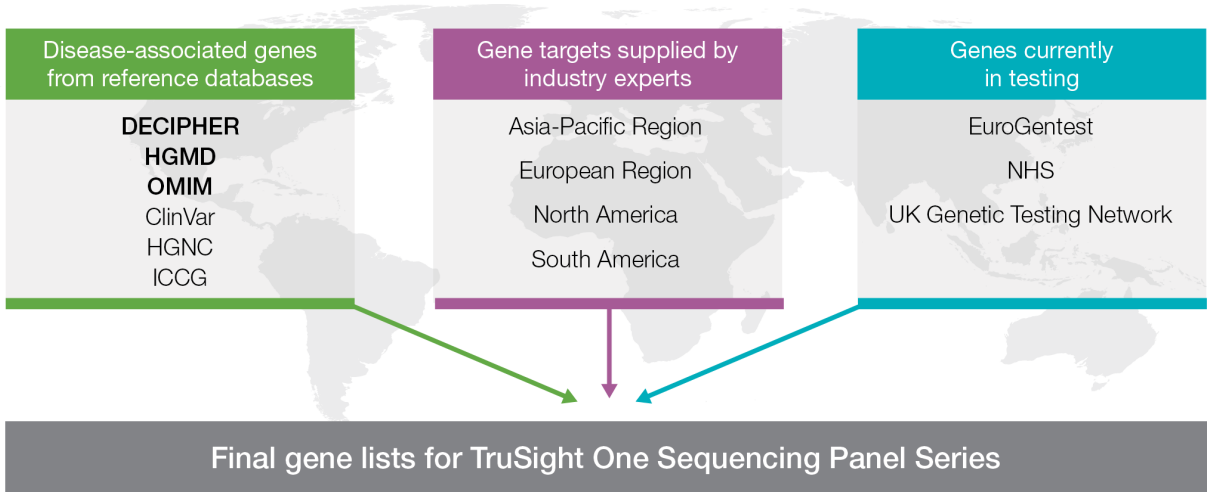

Figure 1: TruSight One Series Global Gene Content Contributors—The TruSight One Panels focus on exonic regions of the genome with known disease-associated mutations. Combining data from multiple public sources ensures that the panels cover all genes currently reviewed in the clinical research setting. Acronyms: HUGO Gene Nomenclature Committee (HGNC), International Collaboration for Clinical Genomics (ICCG), and National Health Service (NHS).

Extensive Content and High Coverage

The TruSight One Probe Design Enables Comprehensive Coverage

The TruSight One Sequencing Panels feature a highly optimized probe design that enables simultaneous analysis of multiple variants. Both panels include over 125,000 probes constructed against the human NCBI37/hg19 reference genome.[6] The 80-mer probes target libraries with ~500 bp mean fragment sizes and ~300 bp insert sizes, enriching a broad footprint of 350–650 bases centered symmetrically around the midpoint of the probe (Figure 2).[7] Therefore, in addition to covering the main exon regions, the panels cover exon-flanking regions, which can provide important biological information (eg, splice sites, regulatory regions).

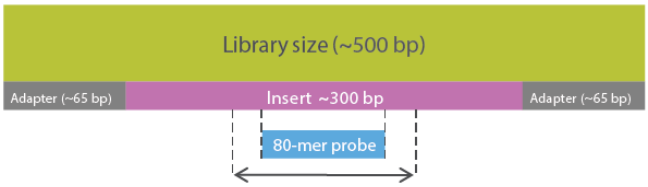

Figure 2: Probe Footprint—With an approximately 500 bp DNA library (insert size of 300 bp), the probe will enrich 350-650 bp centered around its midpoint.

TruSight One probes were built using an iterative design process with functional testing to ensure optimal performance and depth of coverage. The result is ≥ 20× coverage on 95% of the target regions in the panel (Table 1).

High Coverage on a Range of Sequencing Instruments

Whether sequencing on a MiniSeq™, MiSeq™, NextSeq, HiSeq, or NovaSeq™ System, the TruSight One Panels yield consistent high depth of coverage (Table 2). Because the TruSight One Panels focus sequencing on a subset of the genome (eg, genes with phenotype associations), these genes or target regions can be sequenced with a high depth of coverage and deliver high-confidence results.

Streamlined, Fully Supported Workflow

Each step in the TruSight One Panel workflow from library preparation to final data analysis is optimized and integrated to provide a seamless DNA-to-data experiment in just four days (Figure 3).†

Library Preparation and Sequencing

The TruSight One Panel Kits efficiently integrate library preparation and target enrichment steps into one convenient kit (Figure 4). Starting with 50 ng of high-quality DNA, the library prep kits harness the speed of Nextera™ tagmentation chemistry, which eliminates the need for time-consuming mechanical DNA fragmentation. In addition, the kits use a unique pre-enrichment sample pooling strategy that reduces the number of enrichment reactions needed.

Table 1: TruSight One Sequencing Panel Specifications

Parameter	TruSight One	TruSight One Expanded
Cumulative Target Region Size	12 Mb	16.5 Mb
No. of Target Genes	4811	6704
No. of Target Exons	~62,000	~86,000
Probe Size	80-mer	80-mer
No. of Probes	125,395	183,809
Minimum Coverage[a]	> 20×	> 20×
Average Coverage	> 100×	> 100×

a. 95% of target regions typically covered at > 20× (higher percent coverage possible with fewer samples per run)

Table 2: TruSight One Sequencing Panels Instrument Throughput

Instrument and Kit Configuration	TruSight One Panel		
	Reads Passing Filter (M)	Output (Gb)[a]	Samples Per Run[b]
MiniSeq Reagent Kit	22–25	7.5	3
MiSeq Reagent Kit v3	22–25	7.5	3
NextSeq Mid Output Kit	132	40	12
NextSeq High Output Kit	400	120	36
HiSeq Rapid Run Mode[b]	300	90	36
NovaSeq (S1 Flow Cell)	1600	500	96

Instrument and Kit Configuration	TruSight One Expanded Panel		
	Reads Passing Filter (M)	Output (Gb)[a]	Samples Per Run[b]
NextSeq High Output Kit	400	120	24
HiSeq Rapid Run Mode[c]	300	90	18
HiSeq High Output Mode[c]	2000	500	96
NovaSeq (S1 Flow Cell)	1600	500	96

a. HiSeq High Output calculation based on 2 × 125 bp run configuration. All other output calculations based on 2 × 150 bp configuration.
b. Coverage level averages 20× coverage on more than 95% of targets with an average coverage of 100×
c. Single flow cell

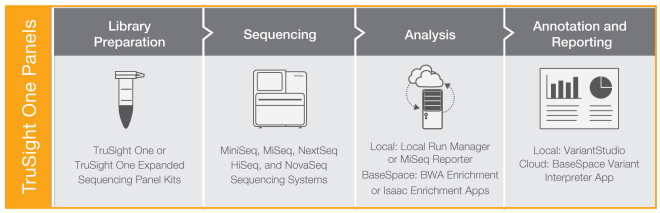

Figure 3: TruSight One Workflow—The Illumina TruSight One Workflow provides a solution for every step from library preparation to data analysis and data reporting.

†Average time for a targeted gene panel. Times may vary according to run configurations.

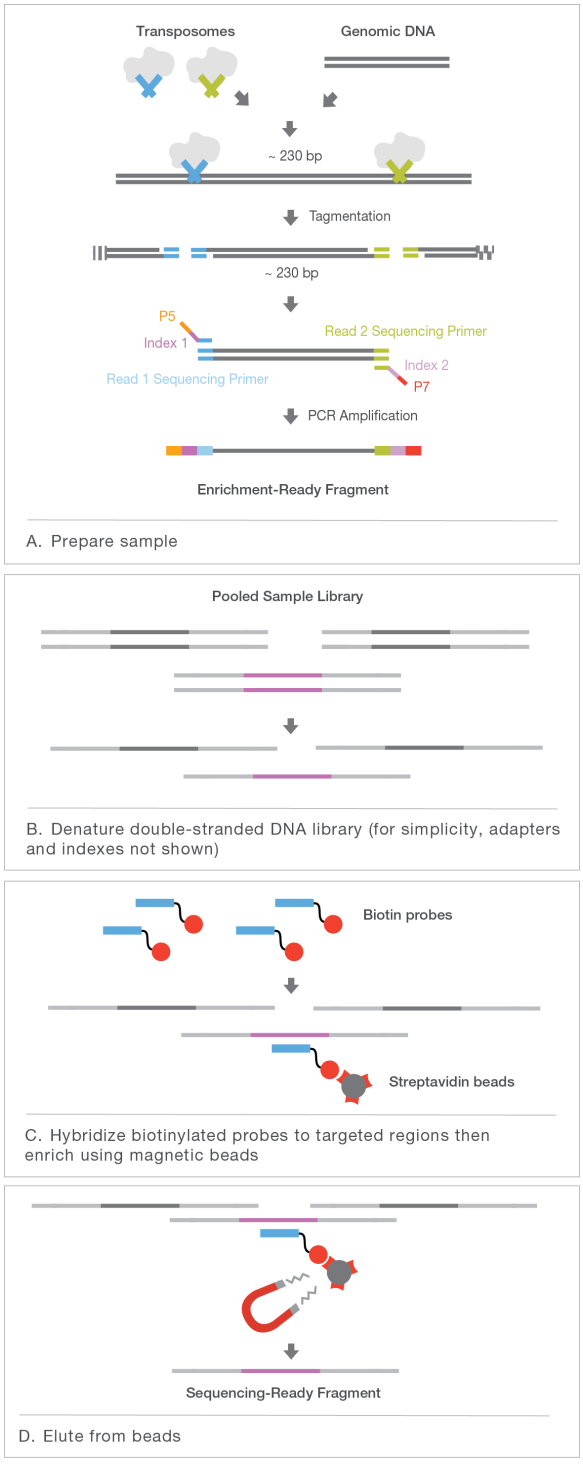

Figure 4: TruSight One and Nextera Enrichment Chemistry—The TruSight One Panel Kits harness Nextera tagmentation chemistry to provide a fast, simple method for enrichment of targeted genes. The workflow combines library preparation and target enrichment steps and can be completed in 1.5 hours.

This strategy uses integrated sample barcodes, which enable pooling of 3–12 samples for a single enrichment pulldown. These efficiencies reduce the overall library preparation time to 1.5 days with ~5.5 hours of hands-on time. Furthermore, master-mixed reagents coupled with plate-based protocols allow simultaneous processing of multiple reactions. Prepared libraries are loaded on to a flow cell for sequencing in the appropriate instrument. Due to the larger total size of the TruSight One Expanded Panel, sequencing on the NextSeq, HiSeq, or NovaSeq Series of Sequencing Systems is recommended.

User-Defined, Flexible Data Analysis and Reporting

Alignment and Variant Calling in BaseSpace Sequence Hub

Sequence data can be streamed directly to BaseSpace Sequence Hub, the Illumina genomics computing environment. Two analysis Apps are available for TruSight One alignment and variant calling in BaseSpace Sequence Hub: BWA Enrichment App, which provides industry-standard Burrows-Wheeler alignment (BWA)[8] and Genome Analysis Toolkit (GATK)[9] variant calling, and Isaac™ Enrichment App,[10] the Illumina pipeline optimized for rapid analysis. After sequencing data is aligned, the BaseSpace apps will calculate a set of alignment-related metrics, including coverage depth, analytical specificity, and uniformity (Figure 5). These metrics, along with variant call metrics, are displayed in user-friendly online reports. Users can export all reports to a PDF file and export raw data tables to a CSV file.

For users who prefer a local data analysis solution, the same data analysis and reporting functionality can be accessed with Local Run Manager software for the MiniSeq System, MiSeq Reporter software for the MiSeq Series, and Onsite BaseSpace Sequence Hub for the NextSeq and HiSeq Systems.

Local Run Manager for the MiniSeq System
MiSeq Reporter for the MiSeq Series of Sequencers
BaseSpace Onsite for all Illumina Sequencers

Biological Interpretation and Reporting

BaseSpace Variant Interpreter is an interpretation and reporting tool designed to simplify and accelerate extraction of biological insight from genomic data. BaseSpace Variant Interpreter can analyze samples imported directly from BaseSpace Sequence Hub or uploaded from a desktop system. Users can identify and interpret disease-relevant variants and generate detailed reports. BaseSpace Variant Interpreter features rich annotation capabilities, an intuitive and highly customizable filtering system, and flexible reporting functionality.

For more detailed information, read the BaseSpace Variant Interpreter data sheet.

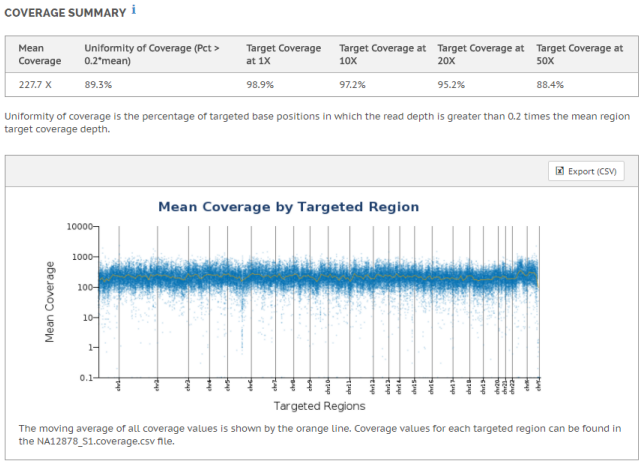

Figure 5: Coverage and Variant Metrics in the Isaac Enrichment App Report—The Isaac Enrichment App displays analysis metrics such as Mean Coverage, Uniformity of Coverage, and generates numerous charts such as a Mean Coverage, Depth of Coverage , and Fragment Length Distribution charts. The Mean Coverage by Targeted Region chart displays coverage levels across the entire target region. The moving average of all coverage values is shown (green line).

For users who prefer a desktop solution, VariantStudio Software enables researchers to explore variant data and quickly identify biologically relevant variants. Using the intuitive software interface, users can easily create reports on subsets of genes from a single TruSight One sequencing run. 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 supports multi-sample comparisons designed to accelerate variant discovery. To support trio and pedigree filtering, the software also provides a collection of filters that identify variants consistent with specified inheritance modes and patterns of disease progression.

Summary

The Illumina TruSight One Series workflow provides a comprehensive DNA-to-data solution for the clinical research environment. Using the TruSight One or the TruSight One Expanded Sequencing Panels, researchers can quickly sequence over 4800 genes with known clinical phenotype association. With flexible filtering options in VariantStudio or BaseSpace Variant Interpreter, the comprehensive TruSight One data set can deliver customized subpanels responsive to specific areas of research and can provide an efficient, effective solution for genetic disease research.

Learn More

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

Learn more about BaseSpace Variant Interpreter at, www.illumina.com/products/by-type/informatics-products/basespace-variant-interpreter.html

For more on VariantStudio Software, visit www.illumina.com/informatics/research/biological-data-interpretation/variantstudio.html

Ordering Information

Product	Catalog No.	TG Catalog No.[a]
TruSight One Sequencing Panel (9 samples)	FC-141-1006	TG-141-1006
TruSight One Sequencing Panel (36 samples)	FC-141-1007	TG-141-1007
TruSight One Expanded Sequencing Panel (36 samples)	FC-141-2007	N/A

a. 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.

References

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