illumina®

TruSight™ One 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 your sequencing portfolio with one assay and one workflow
- **Intuitive, high-powered annotation and reporting**
  Simplify biological interpretation with user-defined gene filtering and report generation

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 panels deliver a comprehensive set of disease-associated target regions designed to cover the most commonly ordered disease gene panels.

The TruSight One and TruSight One Expanded panels provide clinical research labs with an affordable solution for managing a diverse assay portfolio. Investigators can choose to analyze all genes on a panel or focus on a specific subset. With a single assay, labs can expand existing menus, streamline workflows, or create an entire portfolio of sequencing options.

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.

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 panel design targets 16.5 Mb of content, including the original > 4800 genes and ~1900 additional genes with new disease associations in the reference databases.

Extensive content and high coverage

Probe design enables comprehensive coverage

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] 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 percent of the target regions in the panel (Table 1).*

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

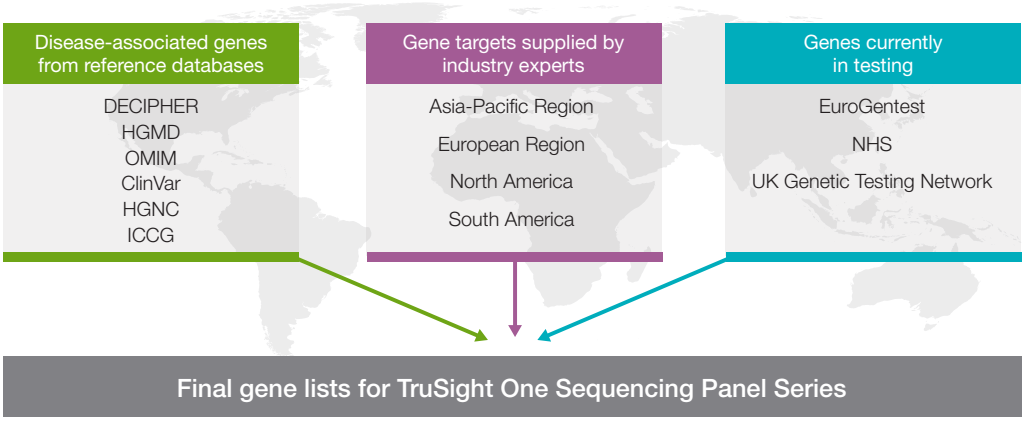

Figure 1: TruSight One Sequencing Panels global gene content contributors—The TruSight One panels focus on exonic regions of the genome with known disease-associated variants. Combining data from multiple public sources makes sure that the panels cover all genes currently reviewed in the clinical research setting. Acronyms: Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources (DECIPHER), HUGO Gene Nomenclature Committee (HGNC), International Collaboration for Clinical Genomics (ICCG), and National Health Service (NHS).

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
Fragment size	150-220 bp	150-220 bp
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)

The 80-mer probes target Illumina DNA Prep with Enrichment libraries with ~300 bp mean fragment sizes and 150-220 bp insert sizes, enriching a broad footprint of bases beyond 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).

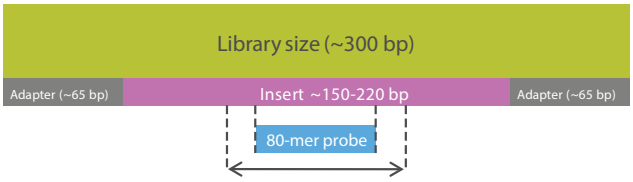

Figure 2: TruSight One probe footprint—With a 300-bp DNA library (insert size of 150-220 bp), the probe will enrich a broad footprint of bases beyond its midpoint.

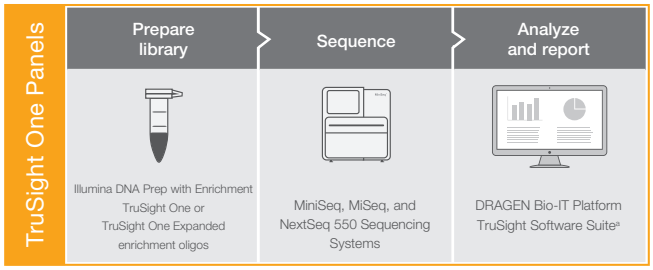

Figure 3: TruSight One workflow—The Illumina TruSight One workflow provides a solution for every step, from library preparation to data analysis and data reporting.
a. TruSight Software Suite available as a paid software-as-a-service (SaaS) platform

High coverage on a range of sequencing instruments

The TruSight One panels are ideal for use on Illumina benchtop sequencers. Table 2 provides recommended sample throughput for the MiniSeq™, MiSeq™, and NextSeq™ 550 Systems. No matter the Illumina sequencing system, the TruSight One panels consistently yield high depth of coverage. 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 (Table 3).

Streamlined, fully supported workflow

Each step in the TruSight One Panel workflow from library preparation to final data analysis is optimized to provide a streamlined DNA-to-data experiment in just two days (Figure 3).† TruSight One Panels are sold as modular kits of enrichment oligos only. Panels integrate seamlessly with the Illumina DNA Prep with Enrichment, (S) Tagmentation Kits and IDT for Illumina DNA UD Indexes (sold separately). The modular approach provides greater flexibility for sample processing.

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

Table 2: Recommended sample throughput for TruSight One Sequencing Panels

	No. of samples per run[a] by instrument and kit configuration			
	MiniSeq System High output	MiSeq System v3 reagents	NextSeq 550 System Mid output	NextSeq 550 System High output
TruSight One Panel	2	3	12	36
TruSight One Extended Panel[b]	1	1	7	24

a. Up to 2 × 150 bp read length; Based on 100× mean coverage of targeted content
b. Higher throughput available on the NovaSeq™ 6000 System (96 samples per run, S1 flow cell) for TruSight One Expanded Panel

Table 3: High depth of coverage with TruSight One Sequencing Panels

	Uniformity of coverage	Target coverage at				Read depth per sample (reads passing filter)
		1×	10×	20×	50×	
TruSight One Panel	95.3%	99.1%	98.3%	97.6%	94.7%	22M
TruSight One Extended Panel	96.8%	99.4%	98.9%	98.6%	97.5%	33M

Simple, efficient library preparation

A key component of Illumina DNA Prep with Enrichment is On-Bead Tagmentation (Figure 4), which uses bead-bound transposomes to mediate a uniform tagmentation reaction. This strategy provides several significant advantages:

- For genomic DNA inputs ≥ 50 ng, accurate quantification of the initial DNA sample is not required as insert fragment size is not affected, saving time and costs associated with kits and reagents
- On-Bead Tagmentation eliminates the need for separate DNA fragmentation steps, saving time and costs associated with related consumables
- For genomic DNA inputs of 50-1000 ng, saturation-based DNA normalization eliminates the need for individual library quantification and normalization steps before enrichment
- Novel 90-minute single hybridization protocol enables enrichment in less than four hours

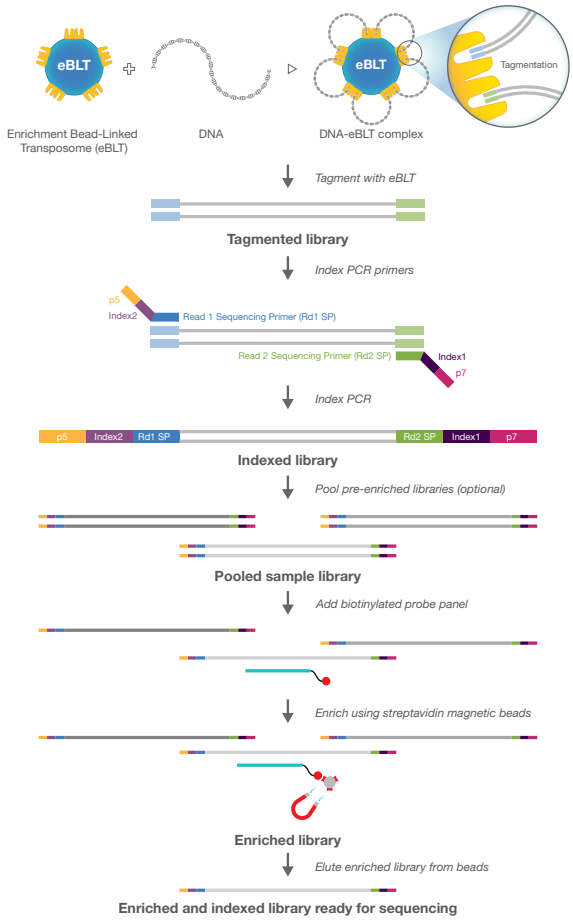

Figure 4: TruSight One and Illumina tagmentation chemistry—The TruSight One enrichment oligos work with Illumina On-Bead Tagmentation chemistry to provide a fast, simple method for enrichment of targeted genes. The workflow combines library preparation and target enrichment steps and can be completed in 1.5 hours.

Fastest Illumina enrichment workflow

Illumina DNA Prep with Enrichment is compatible with liquid-handling systems for library prep automation and produces a workflow with the lowest number of steps and the fastest total workflow time in the Illumina enrichment portfolio.

In addition, the TruSight One workflow uses a unique pre-enrichment sample pooling strategy that reduces the number of enrichment reactions needed. This strategy uses integrated sample barcodes, which enable pooling of up to 12 samples for a single enrichment pulldown. These efficiencies reduce the overall library preparation time to 6.5 hours with ~2 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.

Comprehensive analysis and reporting

For comprehensive TruSight One data analysis, interpretation, and reporting, Illumina offers TruSight Software Suite, a software-as-a-service (SaaS) platform. TruSight Software Suite integrates with BaseSpace™ Sequence Hub to access run monitoring, run metrics, and automated sequencing data upload. It includes cloud-based access to the DRAGEN™ Bio-IT Platform, enabling comprehensive, streamlined secondary and tertiary analysis workflows for NGS.

Variant analysis in TruSight Software Suite

Secondary analysis includes:

- Alignment and variant calling using the DRAGEN platform

Tertiary analysis includes:

- Variant annotation
- Variant filtering and triage
- Variant visualization
- Variant curation
- Variant interpretation and customized reporting

Powered by the DRAGEN platform

TruSight Software Suite is powered by the DRAGEN (Dynamic Read Analysis for GENomics) Bio-IT Platform, providing secondary analysis of genomic data. Fundamental features of the DRAGEN platform address common challenges in genomic analysis, such as lengthy compute times and massive volumes of data. Without compromising accuracy, the DRAGEN platform delivers quickness, flexibility, and cost efficiency, enabling labs of all sizes and disciplines to do more with their genomic data.

Intuitive, high-powered interpretation

TruSight Software Suite displays critical data aggregation, variant visualization, variant curation, and machine-learning tools to promote efficient and informed interpretation.

Results and custom report generation

Interpretation is complete when variants have been identified and curated with known disease associations. Customers can use templates in TruSight Software Suite to customize reports of gene and variant associations relevant to cases (Figure 5). The report can be sent for additional review and approval within the software. For ease of data sharing, reports can be downloaded in a PDF or JSON format.

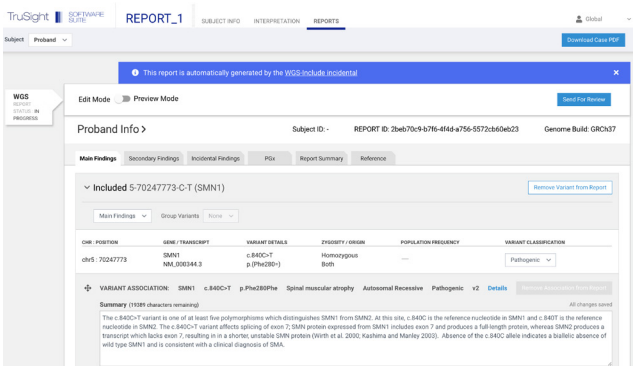

Figure 5: Customizable report generation—TruSight Software Suite offers a template for customizing reports of relevant gene and variant associations.

Secure, compliant environment

TruSight Software Suite is ISO-27001 and ISO-13485 certified and complies with Health Insurance Portability and Accountability Act (HIPAA) (third-party audited) and the principles of the General Data Protection Regulation (GDPR). TruSight Software Suite also offers options to integrate with a lab's single sign-on policy and other security settings.

Summary

The Illumina TruSight One 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 > 4800 genes with known clinical phenotype association. With the intuitive and comprehensive rare disease analysis, interpretation, and reporting solution from TruSight Software Suite, 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 analysis.

Learn more

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

Learn more about TrueSight Software suite at, www.illumina.com/products/by-type/informatics-products/trusight-software-suite.html.

Ordering information

Enrichment oligos	Catalog no.
TruSight One Sequencing Combo (15 samples)	20042621
TruSight One – Enrichment Oligos only (6 enrichment reactions)	20029227
TruSight One Expanded – Enrichment Oligos only (6 enrichment reactions)	20029226
Library preparation kits	**Catalog no.**
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
Indexes	**Catalog no.**
IDT for Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 indexes, 96 samples)	20027213
IDT for Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 indexes, 96 samples)	20027214
IDT for Illumina Nextera™ DNA UD Indexes Set C (96 indexes, 96 samples)	20027215
IDT for Illumina Nextera DNA UD Indexes Set D (96 indexes, 96 samples)	20027216

References

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2. Human Gene Mutation Database (HGMD). www.hgmd.cf.ac.uk/ac/index.php. Accessed November 12, 2020.
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5. TruSight Sequencing Panels. www.illumina.com/products/by-type/clinical-research-products/trusight-one.html. Accessed November 19, 2020.
6. UCSC Genome Browser. genome.ucsc.edu/. Accessed November 12, 2020.
7. Illumina. Optimizing coverage for targeted resequencing technical note. Accessed October 19, 2020.

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