DATA SHEET

TruSight™ One Sequencing Panels

High-performing comprehensive panels targeting disease-associated regions of the exome

- Extensive coverage of up to 6700 disease-associated genes with a minimum of 20× coverage and two panel options
- Single versatile panel replaces iterative testing with one assay and one workflow
- Intuitive, high-powered annotation and reporting with user-defined gene filtering and report generation

illumina®

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] GeneReviews,[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 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

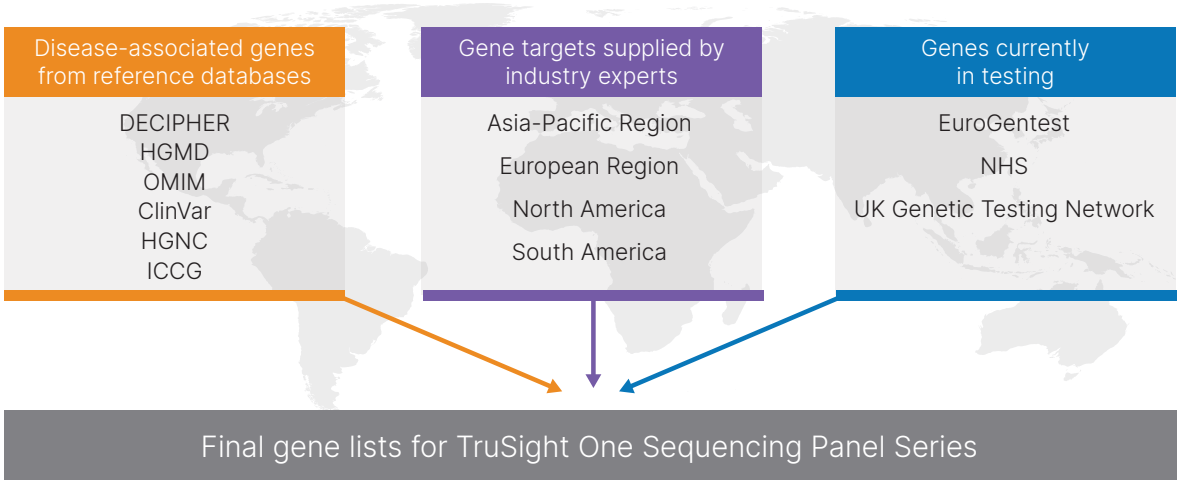

Figure 1: TruSight One Sequencing Panels global gene content contributors—The TruSight One panels focus on exonic regions of the genome with known disease-associated variants. Combining data from multiple public sources makes sure that the panels cover all genes currently reviewed in the clinical research setting.

DECIPHER, Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources; HGNC, HUGO Gene Nomenclature Committee; ICCG, International Collaboration for Clinical Genomics (ICCG); NHS, National Health Service (NHS).

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).*

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]

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).

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).

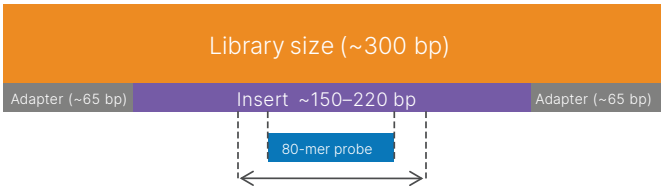

Figure 2: TruSight One probe footprint—With a 300-bp DNA library (insert size of 150–220 bp), the probe will enrich a broad footprint of bases beyond its midpoint.

Compatible with a range of sequencing instruments

The TruSight One panels are ideal for use on Illumina benchtop sequencing systems. Table 2 provides recommended sample throughput for the MiSeq™, NextSeq™ 550, and NextSeq 2000 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).

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

Table 2: Recommended sample throughput for TruSight One Sequencing Panels

	No. of samples per run[a] by instrument and kit configuration			
	MiSeq System v3 reagents	NextSeq 550 System Mid output	NextSeq 550 System High output[c]	NextSeq 2000 System P3 flow cell
TruSight One Panel	3	12	36	96
TruSight One Expanded Panel[b]	1	7	24	66

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.
c. Similar throughput can be achieved with NextSeq 1000 and NextSeq 2000 Systems with P2 flow cells.

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 Expanded Panel	96.8%	99.4%	98.9%	98.6%	97.5%	33M

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 Illumina DNA UD Indexes (sold separately) for library preparation. The modular approach provides greater flexibility for sample processing.

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
- A novel 90-minute single-hybridization protocol enables enrichment in less than four hours

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

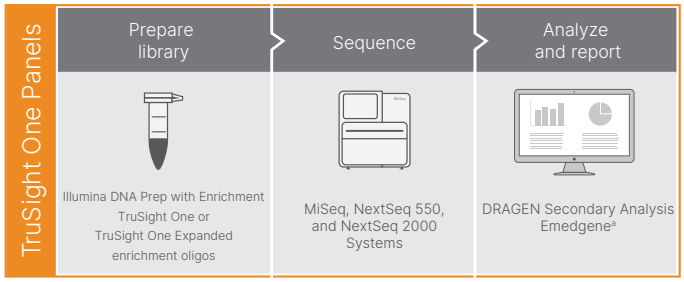

Figure 3: The Illumina TruSight One workflow provides a solution for every step, from library preparation to data analysis and data reporting.

a. Emedgene is available as an optional software platform for germline DNA analysis that is compatible with any library preparation method to enable streamlined, user-defined data interpretation and report generation for research workflows.

Fast enrichment workflow

Illumina DNA Prep with Enrichment is compatible with liquid-handling systems for automating library prep. 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 supports 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.

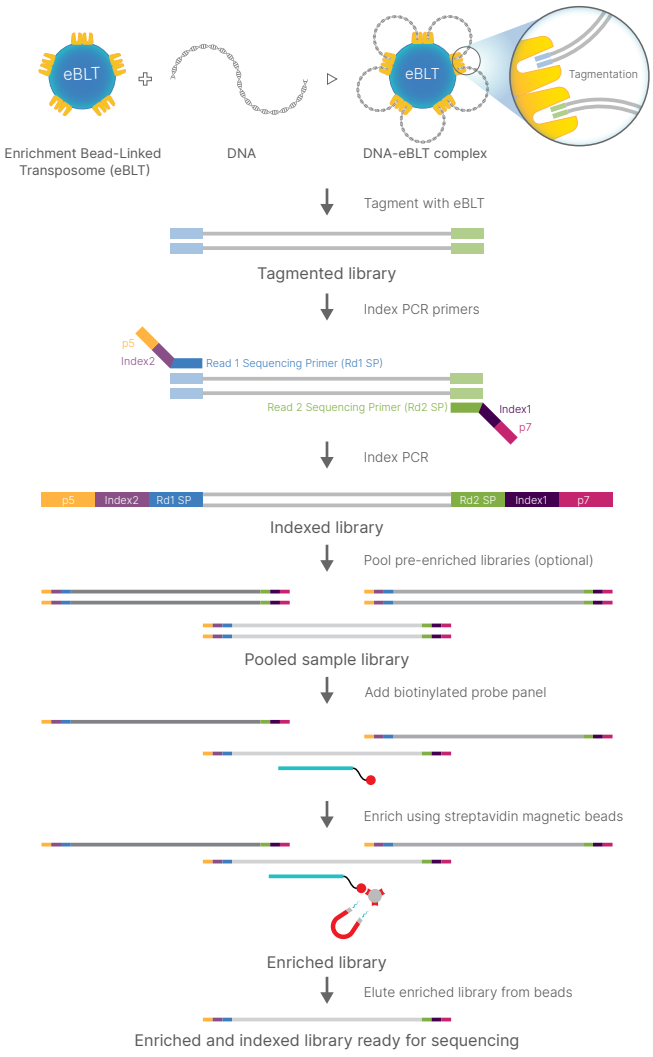

Figure 4: TruSight One and Illumina tagmentation chemistry—The TruSight One enrichment oligos work with Illumina on-bead tagmentation chemistry to provide a fast, simple method for enrichment of targeted genes. The workflow combines library preparation and target enrichment steps and can be completed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

Comprehensive analysis and reporting

For comprehensive TruSight One data analysis, interpretation, and reporting, Illumina offers Emedgene. Emedgene is an optional software tool for research workflows that integrates with BaseSpace™ Sequence Hub and Illumina Connected Analytics to access run monitoring, run metrics, and automated sequencing data upload. It includes cloud-based access to the DRAGEN™ (Dynamic Read Analysis for GENomics) secondary analysis, enabling accurate, comprehensive, and efficient secondary analysis workflows for NGS.

Additional benefits with Emedgene analysis include:

- Streamlined interface with explainable AI (XAI) for highly efficient variant prioritization
- Customizable automation defined by the user for standardization of workflows
- Integrated workflow with DRAGEN secondary analysis and Illumina Connected Analytics for completely automated data movement
- Compatible analysis across hereditary condition applications and assay types (eg, panels, virtual panels, whole-exome sequencing, and whole-genome sequencing)
- Collaboration-ready platform with option to form private, secure networks

Powered by the DRAGEN platform

Emedgene is powered by DRAGEN secondary analysis. 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

Customers report that Emedgene regularly saves 50–75% of their data interpretation time through efficient report generation.[8] Emedgene combines multiple features to power user-defined interpretation, including an always up-to-date annotation and knowledge graph; XAI for transparent, evidence-backed, automated rankings

of potentially causative variant(s) for samples; variant visualization; variant curation; user-defined automation; and more to promote efficient and informed variant interpretation. Emedgene was designed for an intuitive user experience to support users in realizing their optimal impact and efficiency towards their mission.

Panel-based filtering with Emedgene

Emedgene is compatible across hereditary disease assay options for germline analysis, including panels, exomes, and genomes. Additionally, Emedgene enables users to bioinformatically create "virtual panels" from TruSight One Sequencing Panels or other NGS data types, such as whole-exome or whole-genome sequencing (Figure 5). This approach enables labs to standardize multiple assays on a single workflow, simplifying and streamlining lab operations. Also, standardization on a backbone assay removes the need to update and change the assay to add more genes over time and facilitates efficient reanalysis of more genes when needed.

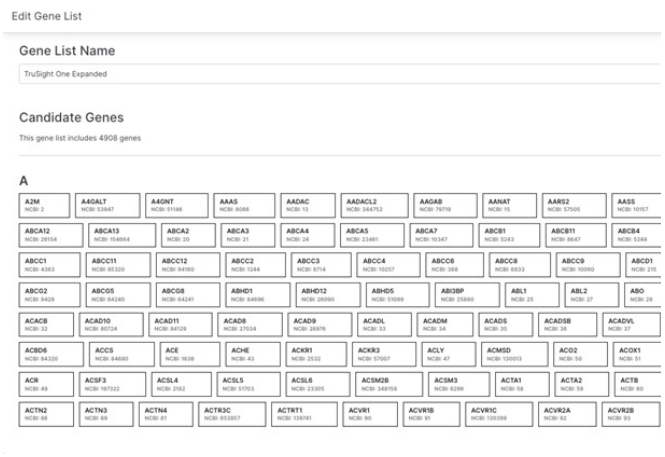

Figure 5: Customizable virtual panels—Emedgene offers the capability to create virtual panels from a subset of genes, either by adding genes individually or as a batch.

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 and other genetic disease insights and report solution from Emedgene, 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

TruSight One Sequencing Panels

Emedgene

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.**
Illumina DNA/RNA UD Indexes Set A, Tagmentation (96 Indexes, 96 Samples)	20091654
Illumina DNA/RNA UD Indexes Set B, Tagmentation (96 Indexes, 96 Samples)	20091656
Illumina DNA/RNA UD Indexes Set C, Tagmentation (96 Indexes, 96 Samples)	20091658
Illumina DNA/RNA UD Indexes Set D, Tagmentation (96 Indexes, 96 Samples)	20091660

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