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TruSight™ Inherited Disease

Using expert-defined content and proven next-generation sequencing technology to efficiently and cost-effectively assess genetic regions known to contain pathogenic mutations in severe, recessive, pediatric-onset diseases.

Highlights

- **Expert-Defined Content**
  Targeting 552 genes, including coding exons, intron-exon boundaries, and regions harboring pathogenic mutations
- **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

Taken individually, Mendelian recessive disorders appear to be uncommon, but when reviewed as a group, these diseases appear within a significant portion of the population. In fact, Mendelian diseases collectively account for ~20% of infant mortalities and ~18% of pediatric hospitalizations[1]. Molecular tests are currently available for a little over 25% of these disorders, contributing to a decline of their appearance. To continue this trend, it is imperative to research screening methods for other recessive inherited disorders.

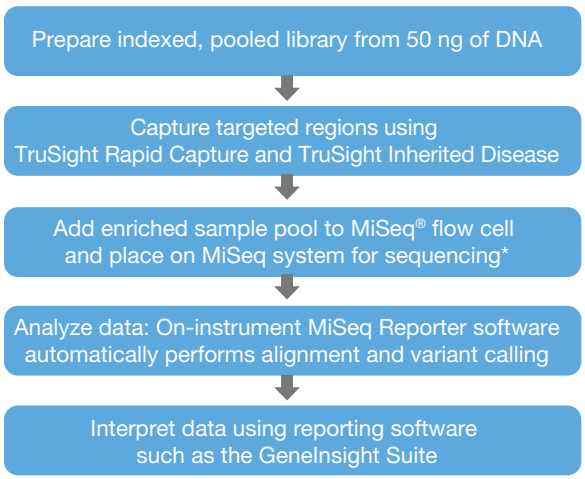
Many of the severe, recessive, pediatric-onset Mendelian disorders are due to pathogenic mutations found in coding exons and intron-exon boundaries. The TruSight Inherited Disease content set provides pre-designed, ready-to-use oligos targeting 552 genes in these specific regions. The content set is compatible with TruSight Rapid Capture Kits that take advantage of Nextera® Rapid Capture technology to offer 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 Inherited Disease and TruSight Rapid Capture enable efficient and reliable analysis of precious samples, while retaining sufficient material for future analyses.

Content Design Strategy

Developed in collaboration with Dr. Stephen Kingsmore and team at Children's Mercy Hospital (CMH) for Pediatric Genomic Medicine in Kansas City, Missouri; Dr. Carol Saunders at CMH, Department of Pathology and Laboratory Medicine; and Dr. Hilger Ropers at the Max Planck Institute, TruSight Inherited Disease targets 552 genes in regions known to harbor pathogenic mutations.

TruSight Inherited Disease was initially based on a 448 gene disease panel designed for the evaluation of severe, recessive childhood disease published by Dr. Kingsmore in Science Translational Medicine[2]. The original content was revised by Dr. Saunders (following ACMG guidelines for testing ultra-rare genetic diseases[3]). Intellectual disability genes were added by Dr. Ropers.

Figure 1: Integrated Workflow



TruSight Inherited Disease 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.

Superior Coverage

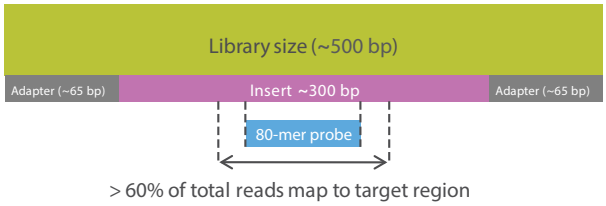
TruSight Inherited Disease features a highly optimized probe set focused on genes with potential involvement in severe, recessive pediatric-onset diseases. The kit includes > 30,000 80-mer probes, each constructed against the human NCBI37/hg19 reference genome. The probe set was designed to enrich for 8,801 exons, spanning 552 genes of interest (Table 1).

TruSight Inherited Disease targets a total of 2.25 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)[4]. This means that the kit provides coverage of exonic and non-coding DNA in exon-flanking regions, on average 50 bp.

Table 1: Coverage Details

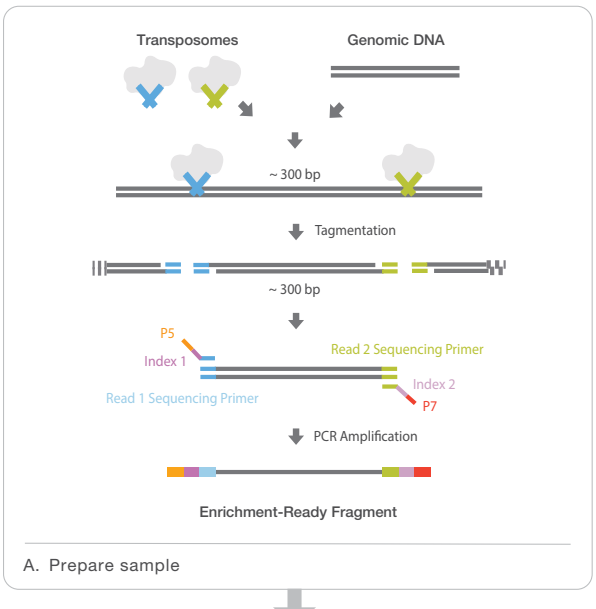
Cumulative target region size	2.25 Mb
Number of target genes	552
Number of target exons	8,801
Probe size	80-mer
Number of probes	~30,000
Recommended mean coverage	100×
Target minimum coverage	20×
Percent exons covered based on coverage metrics	≥ 95%

Figure 2: Probe Footprint



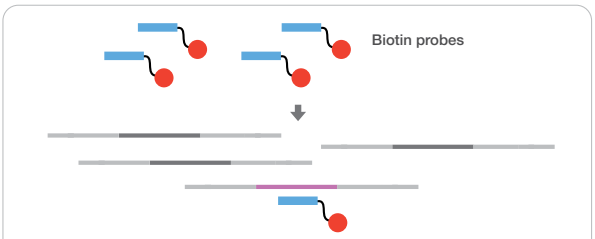
With an approximately 500 bp DNA library (insert size of 300 bp), the probe will enrich 350–650 bp centered around its midpoint.

Figure 3: Integrated TruSight Rapid Capture Workflow

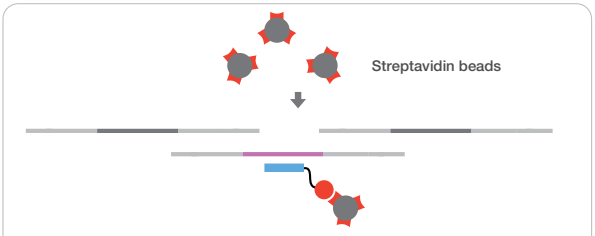


A. Prepare sample

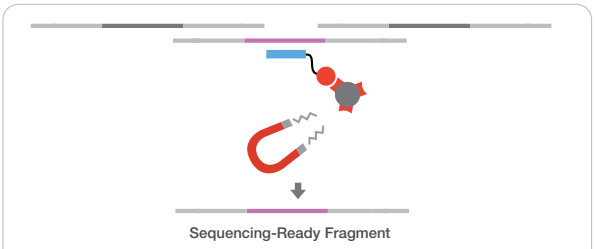
B. Denature double-stranded DNA library (for simplicity, adapters and indexes not shown)



C. Hybridize biotinylated probes to targeted regions



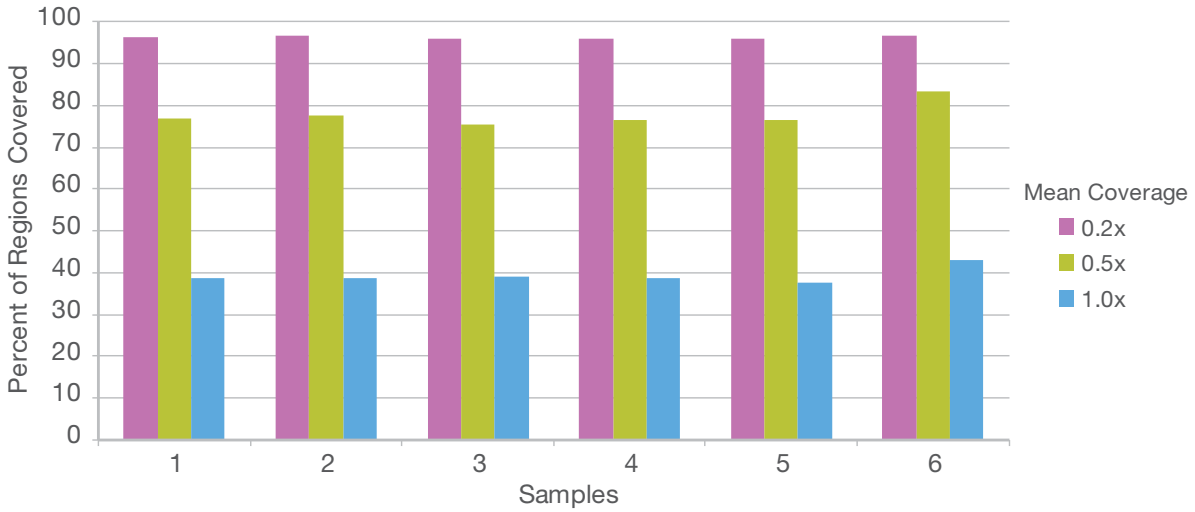
D. Rapid capture using streptavidin beads



E. Elute from beads

The TruSight Rapid Capture workflow provides a fast, simple method for isolating the genes targeted using TruSight Inherited Disease. 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 6 samples with respect to the percentage of targeted regions at varying mean normalized read depths. The 6 samples were prepared and then enriched using the TruSight Rapid Capture Kit along with the TruSight Inherited Disease content set. Samples were sequenced across three MiSeq standard flow cells, generating mean read depths of 100–180× (varying for each sample). Over 95% of bases (> 2.0 Mb) were covered at 0.2× mean coverage for each sample.

Integrated Library Preparation and Enrichment Workflow

TruSight Inherited Disease 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 enrichment 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-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.

Following the TruSight workflow, 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 withoutthe 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 Inherited Disease–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 Inherited Disease 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.

AAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAA
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Ordering Information

Product	Catalog No.	TG Catalog No.*
TruSight Inherited Disease Content Set (4 enrichments)	N/A	TG-141-1005
Rapid Capture Kits		
TruSight Rapid Capture Kit (1 index, 8 samples)	FC-140-1101	TG-140-1101
TruSight Rapid Capture Kit (2 indices, 8 samples)	FC-140-1102	TG-140-1102
TruSight Rapid Capture Kit (4 indices, 16 samples)	FC-140-1103	TG-140-1103
TruSight Rapid Capture Kit (24 indices, 48 samples)	FC-140-1104	TG-140-1104
TruSight Rapid Capture Kit (24 indices, 96 samples)	FC-140-1105	TG-140-1105
TruSight Rapid Capture Kit (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 content sets are available for evaluation purposes prior to executing a supply agreement. Please contact your account manager for more information.

Summary

TruSight Inherited Disease enables researchers to access an expert-defined content set focusing on severe, recessive, pediatric-onset diseases. The optimized probe set provides comprehensive coverage of the targeted regions with high coverage uniformity for identifying variants. Combining this content with TruSight Rapid Capture kits enables a fast, easy workflow, requiring low sample DNA input, generating a highly efficient targeted resequencing solution for capturing these regions known to contain pathogenic mutations, including coding exons intron-exon boundaries.

Learn More

To learn more about TruSight Inherited Disease, TruSight Rapid Capture kits, and Illumina next-generation sequencing technology, visit www.illumina.com/trusight.

References

1. Kingsmore S (2012) Comprehensive carrier screening and molecular diagnostic testing for recessive childhood diseases. PLoS Curr. **4:** e4f9877ab8ffa9.
2. Saunders CJ, Miller NA, Soden SE, Dinwiddie DL, Noll A, et al. (2012) Rapid whole-genome sequencing for genetic disease diagnosis in neonatal intensive care units. Sci Transl Med. **4:** 154ra135.
3. Maddalena A, Bale S, Das S, Grody W, Richards S, et al. (2006) Technical standards and guidelines: molecular genetic testing for ultra-rare disorders. Genet Med. **8:** 735.
4. Optimizing Coverage for Targeted Resequencing Technical Note. www.illumina.com/documents/products/technotes/technote_optimizing coverage_for_targeted_resequencing.pdf

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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