

TruSight™ Cardiomyopathy Sequencing Panel

Using expert-defined content and proven next-generation sequencing technology to efficiently and cost-effectively identify inherited causes of cardiomyopathy.

Highlights

- Expert-Defined Content**
 Developed with the Laboratory for Molecular Medicine and Partners Healthcare Center for Personalized Genetic Medicine to target 46 genes important in the research of inherited cardiomyopathies
- 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

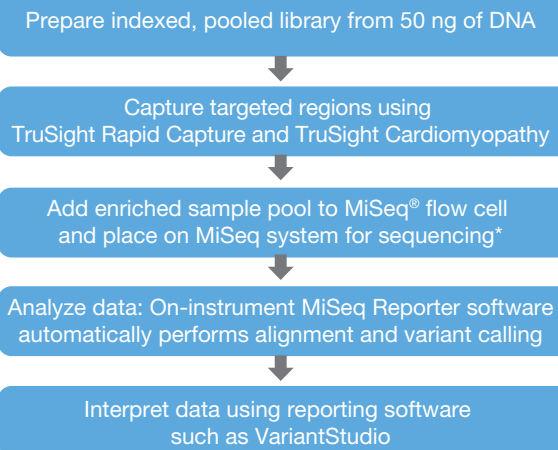
Cardiomyopathy is a disease in which the heart muscle weakens and becomes enlarged, hampering its ability to efficiently pump blood throughout the body and leading, possibly, to heart failure. There are multiple types of cardiomyopathy, and although the exact causes are unknown, risk factors include a genetic component. The TruSight Cardiomyopathy Sequencing Panel provides a comprehensive assessment for suspected genetic causes of all types of cardiomyopathy.

TruSight Cardiomyopathy provides pre-designed, ready-to-use oligos targeting 46 genes important for research of inherited cardiomyopathy. The sequencing panel is compatible with TruSight Rapid Capture that takes 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 Cardiomyopathy 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. Heidi Rehm and team at the Laboratory for Molecular Medicine (LMM) and Partners Healthcare Center for Personalized Genetic Medicine (PHCPGM), Harvard Medical School, TruSight Cardiomyopathy targets genes important in the research of Hypertrophic Cardiomyopathy (HCM), Dilated Cardiomyopathy (DCM), Arrhythmogenic Right Ventricular Cardiomyopathy/Catecholaminergic Polymorphic Ventricular Tachycardia (ARVC/CPVT), and Left Ventricular Noncompaction Cardiomyopathy (LVNC). Content was chosen based on careful review

Figure 1: Integrated Workflow



TruSight Cardiomyopathy 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 throughput, use NextSeq™ flow cells on the NextSeq system or HiSeq[®] flow cells on the HiSeq system.

of the literature and LMM's nine years of experience testing many of these genes¹. Additional content is included from syndromes that present with isolated cardiomyopathies, such as Danon, Fabry, etc.

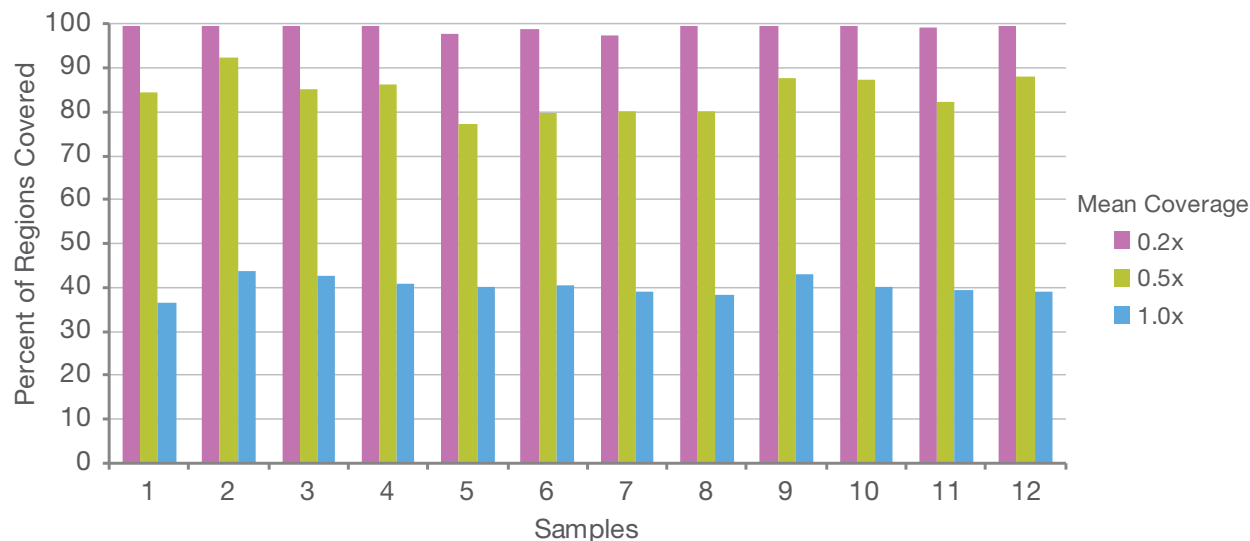
Superior Coverage

The TruSight Cardiomyopathy Sequencing Panel features a highly optimized probe set that delivers comprehensive coverage of genes linked to various cardiomyopathies, starting from only 50 ng of DNA input. The kit includes ~2,600 80-mer probes, each constructed against the human NCBI37/hg19 reference genome. The probe set was designed to enrich for 1,020 exons, spanning 46 genes of interest (Table 1).

TruSight Cardiomyopathy targets a total of 246 Kb 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)². This means that the kit provides coverage of exonic and non-coding DNA in exon-flanking regions, on average 50 bp.

AGAATGATAACAGTAACACACTTCTGTGTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
 TCAACGTACCCTAACGAACGTATCAATTAAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
 CGACGAAAGAAATGATAACAGTAACACACTTCTGTGTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
 AACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
 AGAATGATAACAGTAACACACTTCTGTGTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
 GATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
 CGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCCTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC

Figure 4: High Coverage Uniformity



Coverage uniformity is given for 12 samples with respect to the percentage of targeted regions at varying mean normalized read depths. The 12 samples were prepared and then enriched using the TruSight Rapid Capture Kit along with the TruSight Cardiomyopathy sequencing panel. Pooled samples were sequenced across MiSeq standard flow cells, generating mean read depths of 100–600 \times (varying for each sample). Over 95% of bases (~230 Kb) were covered at 0.2 \times mean coverage.

Integrated Library Preparation and Enrichment Workflow

TruSight Cardiomyopathy 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 Rapid Capture 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 without the 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 Cardiomyopathy 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 Cardiomyopathy 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 \times (Figure 4). This uniformity applies to smaller exons (< 150 bp) as well as long coding exons.

