DATA SHEET

TruSight™ Cardio Sequencing Panel

A comprehensive, cost-effective sequencing panel for identifying causal variants implicated in inherited cardiac conditions

- Expert-defined panel offering highly accurate coverage of 174 genes related to 17 inherited cardiac conditions[1]
- Single, integrated workflow fully supported on Illumina sequencing and informatics platforms
- Rapid turnaround time with low input DNA requirements and cost to sequence ~ $1 USD per gene

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Introduction

Sudden cardiac arrest (SCA) is a leading cause of nontraumatic mortality in the United States.[2-6] At least 25% of SCA events have a genetic component and can be classified as inherited cardiac conditions (ICCs).[2-6] Current genetic assays for studying ICCs are expensive and limited in scale and scope, minimizing their potential for understanding the underlying genetic variation.

The TruSight Cardio Sequencing Panel leverages next-generation sequencing (NGS) to offer comprehensive, accurate, and cost-effective genetic profiling of genes related to ICCs. Using TruSight Cardio, researchers can profile 174 genes that span 17 ICCs, at approximately $1 USD per gene.*

Comprehensive panel design

The TruSight Cardio Sequencing Panel content was expertly selected in collaboration with researchers at the Imperial College of London and the National Heart Center, Singapore. The panel was designed to focus on those ICCs most impacted by a genetic predisposition. Covered cardiac conditions include cardiomyopathies, arrhythmias, aortopathies, and more (Table 1).

The TruSight Cardio panel includes well-characterized core genes (Table 2) and emerging genes. Core genes have well-established links to cardiac conditions. Emerging genes are defined as those genes with demonstrated, but not necessarily understood, connections to cardiac conditions. The emerging gene list includes the 364 exons of the Titin gene (*TTN*) implicated in 25% of the genetically originating dilated cardiomyopathy (DCM) cases.[7]

For a complete TruSight Cardio gene list, visit support.illumina.com/downloads/trusight-cardio-product-files

Table 1: Inherited cardiac conditions covered by the TruSight Cardio Sequencing Panel

Cardiac condition	No. of genes covered
Aortic Valve Disease	3
Marfan Syndrome	3
Loeys-Dietz Syndrome	4
Short QT Syndrome	4
Catecholaminergic Polymorphic Ventricular Tachycardia	6
Familial Hypercholesterolemia	7
Restrictive Cardiomyopathy	9
Non-Compaction Cardiomyopathy	10
Noonan Syndrome	11
Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC)	11
Brugada Syndrome	13
Structural Heart Disease	15
Long QT Syndrome	15
Familial Aortic Aneurysm	16
Familial Atrial Fibrillation	21
Hypertrophic Cardiomyopathy	47
Dilated Cardiomyopathy	59

The TruSight Cardio Sequencing Panel uses NGS for genetic profiling of 174 genes with known associations to 17 ICCs. For a complete TruSight Cardio Sequencing Panel gene list, visit support.illumina.com/downloads/trusight-cardio-product-files

TruSight Cardio captures all *TTN* exons

The most common genetic cause of DCM cases are truncated variants of Titin (*TTN*),[7] the largest human gene.[8] Professor Cook and colleagues described the specific features of *TTN* truncations that discriminate pathogenic from benign variation using a *TTN* assay.[7] The TruSight Cardio Sequencing Panel, which is partly based on this *TTN* assay, efficiently captures all 364 *TTN* exons as noted in the Locus Reference Genomic (LRG) sequence annotation for *TTN*.[9]

* Based on cost of consumables only.

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Table 2: Performance of core genes screened by the TruSight Cardio Sequencing Panel

Gene	% Bases covered at ≥ 10×	% Regions covered at > 10×	% Bases covered at ≥ 20×	% Regions covered at > 20×
ACTA2	100.0	100.0	98.1	100.0
CACNA2D1	100.0	100.0	100.0	100.0
DSC2	100.0	100.0	100.0	100.0
DSG2	100.0	100.0	100.0	100.0
DSP	100.0	100.0	100.0	100.0
ELN	100.0	100.0	100.0	100.0
FBN1	100.0	100.0	99.5	100.0
GJA5	100.0	100.0	100.0	100.0
KCNE1	100.0	100.0	100.0	100.0
KCNE2	100.0	100.0	100.0	100.0
KCNH2	98.7	100.0	98.2	100.0
KCNJ2	100.0	100.0	100.0	100.0
KCNQ1	97.8	100.0	92.6	100.0
DKRAS	100.0	100.0	100.0	100.0
LDLR	100.0	100.0	100.0	100.0
LMNA	100.0	100.0	99.7	100.0
MYBPC3	100.0	100.0	99.6	100.0
MYH6	100.0	100.0	100.0	100.0
MYH7	100.0	100.0	100.0	100.0
NOTCH1	100.0	100.0	98.8	100.0
PKP2	100.0	100.0	100.0	100.0
PTPN11	100.0	100.0	100.0	100.0
RAF1	100.0	100.0	100.0	100.0
RYR2	100.0	100.0	100.0	100.0
SCN5A	100.0	100.0	99.3	100.0
SOS1	100.0	100.0	100.0	100.0
TGFBR1	95.2	100.0	93.9	100.0
TGFBR2	100.0	100.0	100.0	100.0
TNNI3	100.0	100.0	100.0	100.0
TNNT2	100.0	100.0	99.3	100.0
TNN	100.0	100.0	99.9	99.9

Core genes listed here are known to have well-established links to ICCs. For a complete TruSight Cardio gene list, visit support.illumina.com/downloads/trusight-cardio-product-files
Twelve samples were sequenced using the TruSight Cardio Sequencing Panel run on the MiSeq System. PCR duplicates and highest and lowest performing samples were removed from analysis. Performance was calculated from average counts per base (bases covered) and counts per regions (regions covered) across the remaining 10 samples.

Proven technology

Approaches investigating the underlying causes of ICCs can be constrained by tedious workflows, multiple vendors, and limited assay targets that provide an incomplete representation of the conditions. Variants found in ICCs are often highly unique and not always found in the same locations, even within families, and have variable degrees of penetrance.

The TruSight Cardio Sequencing Panel uses NGS technology to provide fast, accurate, and comprehensive genetic profiling of ICCs at a low cost. TruSight Cardio and NGS assess multiple genes in a single assay with a simplified workflow amenable to the clinical research laboratory. This efficiency eliminates the need to employ multiple iterative single-gene tests to identify causative variants, reducing costs and saving time. The comprehensive coverage of the TruSight Cardio Sequencing Panel coupled with high-performance sequencing using Illumina NGS systems offer a broader view into the genetic origin of ICCs.

Fully supported, complete research panel

The TruSight Cardio Sequencing Panel is part of a complete workflow that is fully supported on the MiniSeq™, MiSeq™, MiSeqDx™ (in research mode), NextSeq™ 500, NextSeq 550, NextSeq 1000, and NextSeq 2000 sequencing systems. The panel analyzes variants within exons in 174 genes using 50 ng of DNA per sample (Table 3). Throughput rates are up to 12 samples per run on the MiniSeq and MiSeq systems and up to 96 samples per run on the NextSeq systems.

TruSight Cardio performance includes > 99% of bases sequenced at a depth of coverage of 20× or greater, and an average depth of coverage of 300× (Table 4). At approximately $1 USD per gene, the TruSight Cardio Sequencing Panel enables laboratories to gain a deep understanding of the underlying biology related to ICCs.

Table 3: TruSight Cardio coverage details

Feature	Description
Cumulative target region size	575 kb
Number of target genes	174
Probe size	80-mer
Target minimum coverage depth	20×
Average mean coverage depth	300×
Coverage uniformity (% of targets covered at > 0.2× mean)	> 95%
Read length	2 × 150 bp
Samples per MiSeq System run (v2 chemistry)	Up to 12
Samples per NextSeq 550 System run (mid output chemistry)	Up to 96
Samples per NextSeq 1000 or NextSeq 2000 System run (P2 chemistry)	Up to 384

How it works

TruSight Cardio leverages the speed of Illumina DNA Prep with Enrichment chemistry for a single, integrated library preparation and enrichment workflow that can be completed in 6.5 hours (Figure 1).

One workflow

TruSight Cardio uses a unique multiplex pre-enrichment sample pooling strategy. This workflow eliminates the need for mechanical DNA fragmentation and reduces the number of enrichments needed for a successful library preparation. This method reduces hands-on time for a high-throughput workflow, saving at least one full day over other currently available enrichment workflows. Furthermore, master-mixed reagents are coupled with a plate-based protocol for simultaneous processing of multiple enrichment reactions.

The TruSight Cardio library preparation process starts with Illumina DNA Prep with Enrichment, which converts input genomic DNA into adapter-tagged libraries without the need for mechanical shearing. This method requires only 50 ng of input DNA and takes less than 3 hours. Integrated sample barcodes allow the pooling of 3 to 12 samples for a single pulldown.

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Table 4: Coverage summary for the TruSight Cardio Sequencing Panel

Sample	Mean region coverage depth	Coverage uniformity (% > 0.2× mean)	Target coverage (1×)	Target coverage (10×)	Target coverage (20×)	Target coverage (50×)
1	370.4×	97.5%	100.0%	100.0%	99.9%	99.1%
2	327.6×	97.6%	100.0%	99.9%	99.9%	98.7%
3	348.2×	97.6%	100.0%	100.0%	99.9%	99.0%
4	369.5×	97.7%	100.0%	100.0%	99.9%	99.1%
5	295.1×	97.7%	100.0%	100.0%	99.9%	98.5%
6	290.8×	97.8%	100.0%	99.0%	99.8%	98.5%
7	317.2×	97.8%	100.0%	100.0%	99.8%	98.9%
8	221.7×	97.7%	100.0%	99.9%	99.7%	96.9%
9	275.7×	97.5%	100.0%	99.9%	99.7%	98.0%
10	241.1×	97.3%	100.0%	99.8%	99.5%	97.2%
11	275.0×	97.5%	100.0%	99.9%	99.7%	98.0%
12	272.6×	97.6%	100.0%	99.9%	99.7%	98.1%

Aggregate summary (BWA enrichment) of a representative run on the MiSeq System with 12 samples using the TruSight Cardio Sequencing Panel. Data on file.[10]

Next, libraries are denatured and targeted regions are hybridized to complementary biotin-labeled probes. The pool is enriched for the desired regions by adding streptavidin beads that bind to the biotinylated probes. Biotinylated DNA fragments bound to the streptavdin-coated beads are magnetically pulled down from the solution. The enriched DNA fragments are then eluted from the beads. This entire process is completed in approximately 6.5 hours, enabling a single technician to process multiple samples simultaneously, without automation.

Perform the sequencing

Prepared libraries are loaded on to a flow cell for sequencing with the MiniSeq, MiSeq, MiSeqDx (in research mode), or NextSeq systems. Simply place the flow cell into the instrument and run. Enrichment analysis can be performed using MiSeq Reporter or in the BaseSpace™ Sequence Hub cloud environment. Variant calls generated from the sequencing data are exported as .vcf files and imported easily into VariantStudio™ software for analysis.

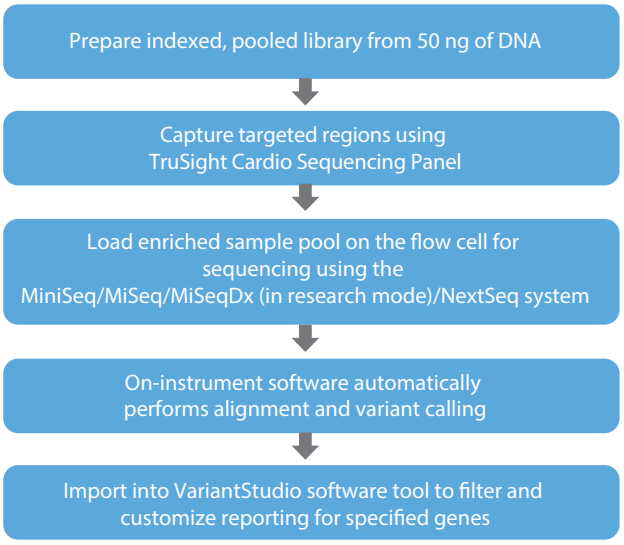

Figure 1: Simple, integrated workflow for library preparation and sample enrichment.

Filtered data analysis

The TruSight Cardio panel can be filtered to isolate a set of genes or regions for analysis and reporting, enabling one assay to represent multiple assays. Simply generate a gene list and select this list when importing .vcf data files from the MiniSeq, MiSeq, MiSeqDx (in research mode), or NextSeq systems into the VariantStudio software. For ease of use, the VariantStudio software offers commonly applied filters, including variant quality, population frequency, functional impact, and known disease association.

Customizable reporting

VariantStudio software enables you to customize reports to meet requirements specific for different diseases of interest and sequencing panels. Multiple report templates can be created and stored for later use. When applying a template to a given sample, simply enter or import sample-specific information from your laboratory information management system (LIMS), combine it with the methodology, a summary of results, and the reported variant categories in the VariantStudio software (Figure 2). Reports, which are linked to the imported sample information, are then exported in PDF or rich-text formats for downstream use.

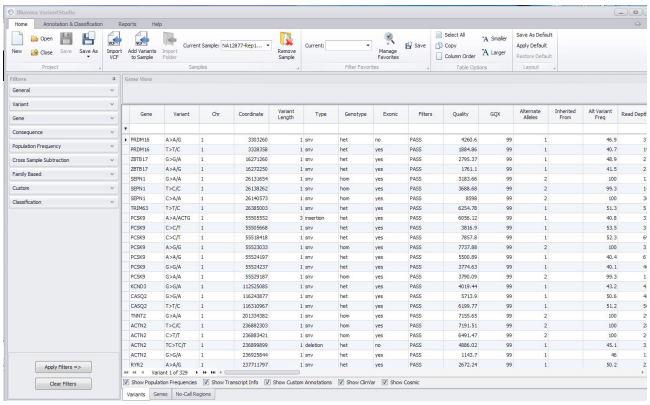

Figure 2: Templates enable customizable reporting.

Accurate data

Whether sequencing on the MiniSeq, MiSeq, MiSeqDx (in research mode), or NextSeq system, researchers can be confident in the quality of the sequencing data generated. Each sample is sequenced with high coverage uniformity across the target region, with 99% of targeted regions covered at a minimum of 20× and a mean coverage of 300× (Table 4).

Summary

The TruSight Cardio Sequencing Panel enables researchers to access an expertly defined panel for analyzing the underlying genetic causes of ICCs. Comprehensive coverage and high uniformity provide accurate identification of 174 genes related to 17 ICCs. The fast, easy workflow requires low sample input for simultaneous multigene analysis, saving time and providing a highly efficient solution to ICC genetic profiling.

Learn More

TruSight Cardio Sequencing Panel,
illumina.com/products/by-type/clinical-research-products/trusight-cardio

MiniSeq, MiSeq, MiSeqDx, NextSeq 500, NextSeq 550, NextSeq 1000, or NextSeq 2000 systems,
illumina.com/systems

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Ordering information

Enrichment oligos	Catalog no.
TruSight Cardio - Enrichment Oligos only (8 enrichment reactions)	20029229
Library preparation kits	**Catalog no.**
Illumina DNA Prep with Enrichment, (S) Tagmentation (16 samples)	20025523
Illumina DNA Prep with Enrichment, (S) Tagmentation (96 samples)	20025524
Illumina DNA Prep, (S) Tagmentation (16 samples)	20025519
Illumina DNA Prep, (S) Tagmentation (96 samples)	20025520
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 DNA/RNA UD Indexes, Set C, Tagmentation (96 indexes, 96 samples)	20027215
IDT for Illumina DNA/RNA UD Indexes, Set D, Tagmentation (96 indexes, 96 samples)	20027216

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