illumina®

TruSight™ Cardio Sequencing Kit

High-performance, affordable, accurate genetic profiling of 174 genes with known associations to 17 different inherited cardiac conditions.

Highlights

- Expert-defined content
  Developed in collaboration with the National Heart Centre Singapore and Imperial College of London to target 174 genes related to 17 inherited cardiac conditions[1]
- High-performing assay
  99% of the targeted regions covered at a depth of at least 20×
- Affordable, robust solution
  Cost to sequence is ~ $1 USD per gene; fully supported with Illumina industry-leading sequencing technology

Introduction

Sudden cardiac arrest (SCA) is a leading cause of nontraumatic mortality in the United States.[2,3] At least 25% of SCA events have a genetic component and can be classified as inherited cardiac conditions (ICCs).[2,3] Understanding and identifying the genetic components are crucial to disease prognosis, therapy choice, and, possibly, outcome. Unfortunately, current genetic assays for ICCs profile only a few genes at a time, making them expensive, tedious, and limited in scale and scope, minimizing their potential. Next-generation sequencing (NGS) technology and the TruSight Cardio Sequencing Kit overcome these challenges by offering affordable, accurate, comprehensive 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 content design strategy

Genes for the TruSight Cardio Sequencing Kit were expertly selected 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 content includes well-characterized core genes (Table 2) and emerging genes. Core genes are those with 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.[4] For a complete TruSight Cardio gene list, visit www.illumina.com/cardio.

Table 1: TruSight One Sequencing Panel specifications

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 (CPVT)	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 Kit uses NGS for genetic profiling of 174 genes with known associations to 17 ICCs. For a complete gene list, visit www.illumina.com/cardio.

Superior technology

Current investigational efforts into underlying causes of ICCs are often artificially constrained by arduous workflows, multiple vendors, and limited assay targets that do not provide a complete 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 Kit takes advantage of NGS technology to provide faster, more accurate, and more comprehensive genetic profiling of ICCs at a lower cost. Unlike other methods, such as PCR and Sanger sequencing, 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. By offering a broader view into the genetic origin of ICCs, the TruSight Cardio Sequencing Kit and Illumina NGS platforms offer the potential to unveil ICCs missed by traditional methods.

*Based on cost of consumables only.

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

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
KRAS	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
TTN	100.0	100.0	99.9	99.9

Twelve samples were sequenced using the TruSight Cardio Sequencing Kit run on the MiSeq™ System. PCR duplicates and highest and lowest performers 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.

Fully supported, complete solution

The TruSight Cardio Sequencing Kit is part of a complete workflow that is fully supported on the MiSeq™, MiSeqDx™ (run in research mode only), and NextSeq™ 500 Sequencing Systems. The kit 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 MiSeq and MiSeqDx Systems and up to 48 samples per run on the NextSeq 500 System.

TruSight Cardio performance boasts > 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 Kit enables laboratories to focus on gaining a deeper understanding of the underlying biology related to ICCs.

Table 3: TruSight Cardio coverage details

Cumulative Target Region Size	0.572 Mb
Number of Target Genes	174
Probe Size	80-mer
Target Minimum Coverage Depth	20×
Average Mean Coverage Depth	300×
Uniformity of Coverage (% 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 500 System run (mid-output chemistry)	Up to 48

Table 4: Coverage summary for the TruSight Cardio Sequencing Kit

Sample	Mean Region Coverage Depth	Uniformity of Coverage (% > 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%	99.9%	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 Kit.

TruSight Cardio Captures *TTN* Exons

Dilated cardiomyopathy (DCM), a common cause of heart failure, is often genetic in origin. Professor Stuart Cook and colleagues found that the most common genetic cause of DCM cases are truncated variants of Titin (*TTN*),[1] the largest human gene.[2] The study described the specific features of *TTN* truncations that discriminate pathogenic from benign variation using a *TTN* assay.[1] Based in part on the *TTN* assay used in this study, the TruSight Cardio Sequencing Panel efficiently captures all 364 *TTN* exons as noted in the Locus Reference Genomic sequence (LRG) annotation for *TTN*.[3]

References

1. Roberts AM, Ware JS, Herman DS, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titn truncations in health and disease. *Sci Transl Med.* 2015;7(270):p 270ra6.
2. Herman DS, Lam L, Taylor MR, et al. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med.* 2012;366(7):619-28.
3. Locus Reference Genomic, LRG_391 www.lrg-sequence.org. Accessed July 9, 2018.

How It Works

TruSight Cardio leverages the speed of Nextera™ library preparation technology for a single, integrated library preparation and enrichment workflow that can be completed in just 1.5 days (Figure 1).

One workflow

TruSight Cardio uses a unique multiplex pre-enrichment sample pooling strategy that 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 that saves at least 1 full day over all 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 Nextera tagmentation, 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. Next, libraries are denatured and biotin-labeled probes specific to the targeted region are used for hybridization. The pool is enriched for the desired regions by adding streptavidin beads that bind to the biotinylated probes. Biotinylated DNA fragments bound to the streptavidin-coated beads are magnetically pulled down from the solution. The enriched DNA fragments are then eluted from the beads and hybridized for a second capture. This entire process is completed in only 1.5 days, 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 MiSeq, MiSeqDx, or NextSeq Sequencing Systems. Simply place the flow cell into the instrument and run. Enrichment analysis can be performed using MiSeq Reporter or in the BaseSpace™ cloud environment. Variant calls generated from the sequencing data are exported as .vcf files and imported easily into VariantStudio™ for analysis.

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 MiSeq, MiSeqDx, or NextSeq System into VariantStudio. For ease of use, VariantStudio software offers commonly applied filters, including variant quality, population frequency, functional impact, and known disease association.

Customizable Reporting

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

Accurate Data

Whether sequencing on the MiSeq, MiSeqDx, 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 Kit enables researchers to access an expert-defined content set for analyzing the underlying genetic causes of ICCs. Comprehensive coverage and high uniformity provide accurate identification of over 170 genes related to ICCs. The fast, easy workflow requires low sample input for simultaneous multigene analysis, saving time and money and providing a highly efficient solution to ICC genetic profiling.

Learn More

For a complete gene list and to learn more about the TruSight Cardio Sequencing Kit, visit www.illumina.com/cardio.

For more on the MiSeq, MiSeqDx or NextSeq 500 Systems, visit www.illumina.com/systems.html

Ordering Information

Product	Catalog No.
TruSight Cardio Sequencing Kit for MiSeq and MiSeqDx Systems, v2 chemistry (12 samples)	FC-141-1010
TruSight Cardio Sequencing Kit for NextSeq 500 System, mid-output chemistry (48 samples)	FC-141-1011

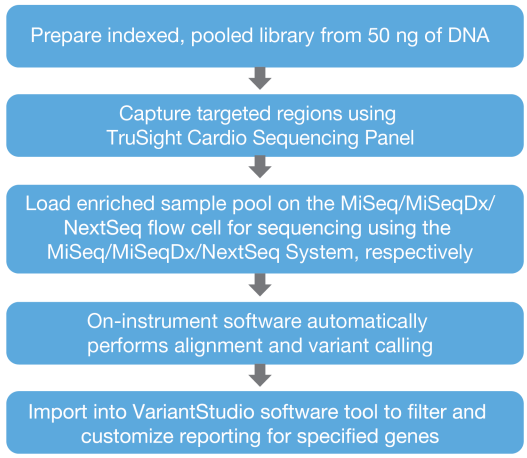

Figure 1: Simple, integrated library preparation and enrichment workflow

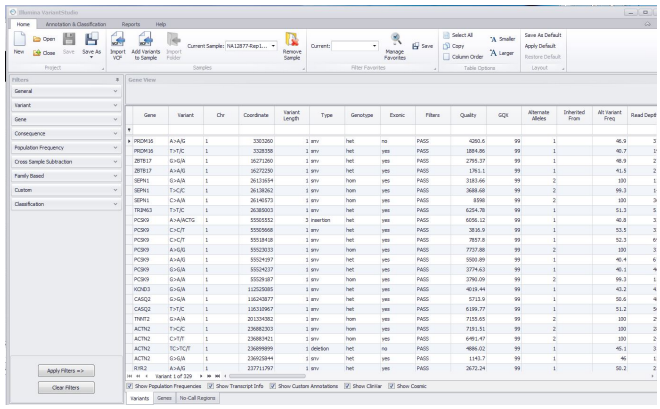

Figure 2: Templates enable customizable reporting

References

1. Pua CJ, Bhalshankar J, Miao K, et al. Development of a comprehensive sequencing assay for inherited cardiac condition genes. *J Cardiovasc Transl Res*. 2016;9(1):3-11.
2. Zipes DP, Wellens HJ. Sudden cardiac death. *Circulation.* 1998;98 (21):2334-2351.
3. Deo R, Albert CM. Epidemiology and genetics of sudden cardiac death. *Circulation.* 2012;125(4):620-637.
4. Roberts AM, Ware JS, Herman DS, et al. Integrated allelic, transcriptional, and phenomic dissection of the cardiac effects of titn truncations in health and disease. *Sci Transl Med.* 2015;7(270):p 270ra6.

Illumina, Inc. • 1.800.809.4566 toll-free (US) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com