HumanCVD Genotyping BeadChip

The HumanCVD BeadChip features more than 48,000 markers targeting candidate genes associated with cardiovascular disease and other associated phenotypes. This 12-sample Infinium® BeadChip supports cost-effective, high-throughput studies on disorders of the heart and blood vessels.

HumanCVD BeadChip Highlights

- **High Data Quality:**
  Achieve > 99% average call rates and > 99.9% reproducibility
- **Comprehensive CVD Coverage:**
  Capture variation, including variants with low odds ratios, without increasing sample
- **Simple Workflow:**
  Use the PCR- and ligation-free Infinium II Assay protocol
- **High-Throughput Format:**
  Interrogate up to 12 samples in parallel

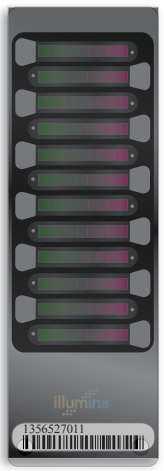
Introduction

Cardiovascular disease (CVD) is responsible for one-third of all deaths worldwide and has been shown to have significant heritability[1,2]. Numerous CVD studies suggest that many variants associated with CVD have low odds ratios (< 1.5), suggesting that very large sample sizes are required to detect variation using a genome-wide association approach. To enable researchers to capture this variation without the need for large sample groups, Illumina has developed the first CVD gene-centric array. Designed in collaboration with researchers from the Institute of Translational Medicine and Therapeutics, the Broad Institute, SeattleSNPs, and the Candidate-gene Association Resource (CARe), the HumanCVD BeadChip offers researchers high-quality, focused CVD content that will accelerate their studies and reduce overall project cost.

The HumanCVD BeadChip features 48,742 markers that capture genetic diversity across approximately 2,100 genes associated with CVD. BeadChip content is derived from published scientific literature, CVD pathway analysis, and recent whole-genome analysis data sets. The rationally selected marker set offers comprehensive coverage of genes in pathways underpinning primary and secondary vascular disease processes such as blood pressure, insulin resistance, metabolic disorders, dyslipidemia, and inflammation.

The HumanCVD BeadChip is a multi-sample genotyping panel powered by Illumina's Infinium II Assay. This revolutionary assay provides the industry's highest call rates, allows for flexible content deployment, and enables the detection and measurement of copy number variation. In addition, the assay's PCR-free single-tube sample preparation protocol[3,4] significantly reduces labor and potential sample handling errors. The multi-sample format allows researchers to interrogate up to 12 samples in parallel, minimizing experimental variability and lowering project cost.

Figure 1: HumanCVD BeadChip



This multi-sample BeadChip allows researchers to interrogate up to 12 samples in parallel and assay 48,742 markers per sample.

HumanCVD BeadChip Content

HumanCVD BeadChip content targets regions associated with a range of cardiovascular, metabolic, and inflammatory syndromes, which can be divided into three broad groups[5]. Group 1 loci comprise approximately 435 genes and regions with a high likelihood of functional significance based on the literature. Tag SNPs derived from the HapMap Project and SeattleSNPs data sets were selected to capture all known variants in these regions with MAFs > 0.02 at an $r^2 \geq 0.8$. Group 2 loci, also based on HapMap Project and SeattleSNPs data, are a set of 1,349 loci potentially involved in cardiovascular pathologies and other metabolic, heart, lung, blood, or circadian phenotypes. Tag SNPs with MAFs > 0.05 and $r^2 \geq 0.5$ were selected to cover all known variants in these regions. Group 3 loci comprise approximately 232 larger genes which were of lower interest to the CVD consortium investigators. Only nonsynonymous SNPs and known functional variants with a MAF ≥ 0.01 were included from these regions.

To empower meta-analysis with data from the literature, the majority of SNPs highly associated with CVD and those that have known or putative functionality are included on the HumanCVD BeadChip. Where possible, nonsynonymous SNPs and known functional variants with MAFs ≥ 0.01 were selected for all genes of interest.

Finally, the HumanCVD BeadChip contains more than 1,500 admix-

Table 1: HumanCVD BeadChip Performance And Specifications

Category	Specification	CEU	CHB+JPT	YRI	TOtal
Call Rate	> 99%	99.84%	99.85%	99.87%	99.79%
Reproducibility	> 99%	99.99 %	99.99%	100%	99.99%
Mendelian Inconsistencies	< 0.01%	0.006%	0.01%	N/A	0.001%

Results based on 284 HapMap samples including 59 trios and 15 replicates.

ture and ancestry informative SNP markers (AIMs) for African versus European ancestry. It also features 400 AIMs for regional European ancestral populations. See Table 2 for a description of HumanCVD BeadChip probe categories and coverage.

High Density

The HumanCVD BeadChip has an average density of 36.5 SNPs per locus across Group 1 loci, which are highly associated with CVD, providing more than twice the density of standard whole-genome genotyping arrays. This higher density offers greater resolution for detecting potentially causal variants, including variants with low odds ratios, without the need to increase sample size.

Validated Markers

The HumanCVD BeadChip was validated using 117 HapMap samples (Table 1). The Caucasian (CEU) and Han Chinese , Japanese (CHB+JPT), and Yoruban (YRI) populations contain 30,774, 28,640, and 35,600 SNPs, respectively, with MAFs ≥ 0.05. The HumanCVD BeadChip also offers coverage of rare variants with MAFs between 0.01 and 0.05 identified from HapMap allele frequencies (Figure 2). BeadChip markers target 4,815, 4,215, and 4,918 rare SNPs in the CEU, CHB+JPT, and YRI populations, respectively. A significant portion of SNPs on the HumanCVD BeadChip are derived from SeattleSNPs[6], the literature, and recent reseqeuncing efforts[7], offering coverage in populations beyond HapMap.

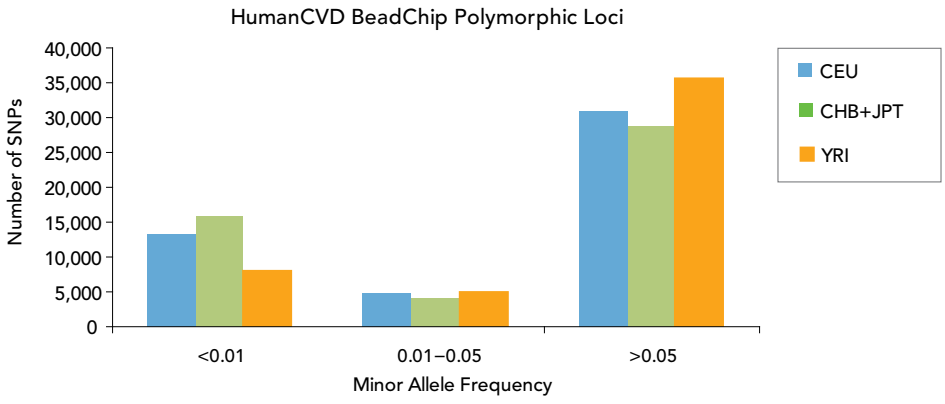
Analysis Simplified

Illumina's BeadStudio analysis software is a powerful and user-friendly tool for analyzing data generated by the HumanCVD Genotyping BeadChip. The Genotyping Module provides automated genotype calling and sample clustering. Powerful visualization tools and quality-control features enable graphical output of data and reports on reproducibility and Mendelian consistency.

Automation

As with most of Illumina's standard DNA Analysis products, an optional Laboratory Information Management System (LIMS) and robotic automation track samples to provide accurate and efficient workflow management and overall project management. This system, custom designed for Infinium Assay workflows, allows labs to maximize their throughput with a completely integrated microarray solution.

Figure 2: Human BeadChip Coverage Of Common And Rare SNPS



HumanCVD BeadChip content provides more than twice the density of standard genotyping arrays across loci associated with CVD.

Table 2: HumanCVD BeadChip Markers Categories

Category	Number of Markers*
Synonymous SNPs	975
Nonsynonymous SNPs	3,470
Non-Genic Markers	10,758
SNPs in Untranslated Regions	1,775
SNPs in Introns	31,313
Ancestry Informative Markers	1,414
Highly Conserved Cross-Species Regions	4,026

* Markers may be counted in multiple categories. Gene region markers are based on NCBI annotation.

Services

Illumina FastTrack Genotyping Services are available to analyze samples in a timely fashion at a reasonable cost. This option allows researchers to acquire high-quality data for limited studies or before purchasing their own equipment.

Summary

The HumanCVD BeadChip contains CVD-focused content that allows researchers to detect variants, even those with low odds ratios, without the need for large sample groups. Offering superior coverage of known and associated CVD loci across multiple populations, this BeadChip empowers the development of better tools for diagnosing and treating this widespread health problem. The HumanCVD BeadChip delivers the industry's highest quality data, allowing researchers to confidently pursue the fastest path to discoveries and publication.

References

1. http://www.who.int/hpr/gs.fs.cvds.shtml
2. Kullo IJ and Ding K (2007) Mechanisms of disease: The genetic basis of coronary heart disease. Nat Clin Pract Cardiovasc Med 4:558–569.
3. Gunderson KL, Steemers FJ, Lee G, Mendoza LG, Chee MS (2005) A genome-wide scalable SNP genotyping assay using microarray technology. Nat Genet 37(5): 549–554.
4. Steemers FJ, Weihua Chang W, Lee G, Barker DL, Shen R, et al. (2006) Whole-genome genotyping with the single-base extension assay. Nat Methods 3(1): 31–33.
5. A complete list of HumanCVD BeadChip SNPs can be found at http://bmic.upenn.edu/cvdsnp
6. http://pga.gs.washington.edu
7. http://egp.gs.washington.edu

Ordering Information

Catalog No.	Product	Description
WG-31-151	HumanCVD Genotyping Kit	Each package contains four BeadChips and reagents for processing 48 samples.
WG-31-152	HumanCVD Genotyping Kit	Each package contains 24 BeadChips and reagents for processing 288 samples.
WG-31-153	HumanCVD Genotyping Kit	Each package contains 96 BeadChips and reagents for processing 1152 samples.
FT-20-113	HumanCVD FastTrack Service Project	Illumina's FastTrack Services require a minimum of 94 samples and 1.5 µg of DNA for each sample.

The HumanCVD Genotyping BeadChip can process twelve samples in parallel and genotype 48,742 loci per sample.

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