Infinium® HumanMethylation450 BeadChip
The ideal solution for affordable, large sample-size genome-wide DNA methylation studies.

**Infinium® HumanMethylation450 BeadChip Highlights**

- **Unique Combination of Genome-Wide Coverage, High-Throughput, and Low Cost**
  Genome-wide coverage using over 450,000 methylation sites per sample at single-nucleotide resolution

- **Unrivaled Assay Reproducibility**
  > 99% reproducibility for technical replicates

- **Simple Workflow**
  PCR-free protocol with the powerful Infinium HD Assay

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**Introduction**

DNA methylation plays an important and dynamic role in regulating gene expression. It allows cells to become specialized and stably maintain those unique characteristics throughout the life of the organism, suppresses the deleterious expression of viral genes and other non-host DNA elements, and provides a mechanism for response to environmental stimuli. Aberrant DNA methylation (hyper- or hypomethylation) and its impact on gene expression have been implicated in many disease processes, including cancer.

To provide cost-effective DNA methylation analysis for a variety of applications, Illumina offers a robust methylation profiling platform consisting of its proven Infinium chemistries on the iScan and HiScan™ SQ systems. Leveraging Infinium HD technology, the HumanMethylation450 BeadChip (Figure 1) offers a unique combination of comprehensive, expert-selected coverage, high throughput, and low price, making it ideal for screening large sample populations such as genome-wide association study (GWAS) cohorts. By providing quantitative methylation measurement at the single-CpG-site level, this assay offers powerful resolution for understanding epigenetic changes.

**Comprehensive Genome-Wide Coverage**

The Infinium HumanMethylation450 BeadChip provides unparalleled, genome-wide coverage featuring comprehensive gene region and CpG island coverage, plus additional high-value content selected with the guidance of methylation experts. Infinium HD technology enables content selection independent of bias-associated limitations often associated with methylated DNA capture methods. As a result, 99% of RefSeq genes are covered, including those in regions of low CpG island density and at risk for being missed by commonly-used capture methods.

Importantly, coverage was targeted across gene regions with sites in the promoter region, 5'UTR, first exon, gene body, and 3'UTR in order to provide the broadest, most comprehensive view of methylation state possible (Figure 2). This multiple-site approach was extended to CpG islands / CpG island regions for which 96% of islands were covered overall, with multiple sites within islands and island shores, as well as those regions flanking island shores (island shelves). Beyond gene and CpG island regions, multiple additional content categories requested by methylation experts were also included:

- CpG sites outside of CpG islands
- Non-CpG methylated sites identified in human stem cells
- Differentially methylated sites identified in tumor versus normal (multiple forms of cancer) and across several tissue types
- FANTOM 4 promoters
- DNase hypersensitive sites
- miRNA promoter regions
- ~ 90% of content contained on Illumina’s HumanMethylation27 BeadChip

**Workflow**

The HumanMethylation450 BeadChip follows a user-friendly, streamlined workflow that does not require PCR. Its low sample input requirement (as low as 500 ng), enables analysis of valuable samples derived from limited DNA sources. HumanMethylation450 BeadChip kits contain all required reagents for performing methylation analyses (except for the bisulfite conversion kit, which is available separately).
Data Integration

Of all the genes represented on the HumanMethylation450 BeadChip, more than 20,000 are also present on the HumanHT-12 v4 Expression BeadChip, permitting combined analysis of global methylation status and gene expression levels. In addition, investigators may integrate methylation data with genotyping data from GWAS studies to better understand the interplay between genotype and methylation state in driving phenotypes of interest.

High-Quality Data

The HumanMethylation450 BeadChip applies both Infinium I and II assay chemistry technology (Figure 3) to enhance the depth of coverage for methylation analysis. The addition of the Infinium II design allows use of degenerate oligonucleotide probes for a single bead type, enabling each of up to three underlying CpG sites to be either methylated or unmethylated with no impact on the result for the queried site.

Illumina scientists rigorously test every product to ensure strong and reproducible performance, enabling researchers to achieve industry-leading data quality.

Precision and Accuracy

Reproducibility has been determined based on the correlation of results generated from technical replicates. The HumanMethylation450 BeadChip showed strong correlation between replicates (r^2~0.99), as well as with the HumanMethylation27 BeadChip and whole-genome bisulfite sequencing (Figure 4).

Sensitivity

By comparing the results of replicate experiments (duplicates of eight biological samples), Illumina scientists have shown that the HumanMethylation450 BeadChip reliably detects a delta-beta value of 0.2 with a lower than 1% false positive rate.

Internal Quality Controls

Infinium HD-based assays possess several sample-dependent and sample-independent controls so researchers have confidence in producing the highest quality data. The HumanMethylation450 BeadChip includes 600 negative controls, which are particularly important in methylation analysis assays since sequence complexity is decreased after bisulfite conversion. The GenomeStudio® Methylation Module Software has an integrated Controls Dashboard where the performance of all controls can be easily monitored.

Integrated Analysis Software

HumanMethylation450 BeadChip data analysis is supported by the powerful and intuitive GenomeStudio Methylation Module, enabling researchers to effortlessly perform differential methylation analysis (Figure 5). The GenomeStudio software features advanced visualization tools that enable researchers to view vast amounts of data in a single graph, such as heat maps, scatter plots, and line plots. These tools and the GenomeStudio Genome Browser display valuable information such as chromosomal coordinates, percent GC, location in a CpG Island, and methylation β values.

Figure 3: Infinium I and II Assay Designs

The HumanMethylation450 BeadChip employs both Infinium I and Infinium II assays, enhancing the breadth of coverage it is able to include. Infinium I assay design employs two bead types per CpG locus, one each for the methylated and unmethylated states. The Infinium II design uses one bead type, with the methylated state determined at the single base extension step after hybridization.
Figure 4: Assay Reproducibility
A: HumanMethylation450 Replicate Correlation

B: HumanMethylation27 vs. HumanMethylation450 Correlation

C: HumanMethylation450 vs. Whole-Genome Bisulfite Sequencing

Using the HumanMethylation450 BeadChip, users can be confident of obtaining consistent, robust data. Representative plots from internal testing show strong replicate correlation (A), as well as strong correlation with the HumanMethylation27 BeadChip (B) and whole-genome bisulfite sequencing (C).

Data generated by the Infinium HD methylation assay are easily compatible with data from other Illumina applications, including gene expression profiling. This enables researchers to perform cross-application analysis such as the integration of gene expression data with HumanMethylation450 BeadChip methylation data.

Summary
The HumanMethylation450 BeadChip’s unique combination of comprehensive, expert-selected coverage, high sample throughput capacity, and affordable price makes it an ideal solution for large sample-size, genome-wide scale DNA methylation studies.

References