Infinium® HumanMethylation450 BeadChip

The ideal solution for affordable, large sample–size genome-wide DNA methylation studies.

HumanMethylation450 BeadChip Highlights

- **Unique Combination of Genome-Wide Coverage, High-Throughput, and Low Cost**
  Over 450,000 methylation sites per sample at single-nucleotide resolution
- **Unrivaled Assay Reproducibility**
  > 98% reproducibility for technical replicates
- **Simple Workflow**
  PCR-free protocol with the powerful Infinium HD Assay
- **Compatibile with FFPE Samples**
  Protocol available for methylation studies on FFPE samples

Introduction

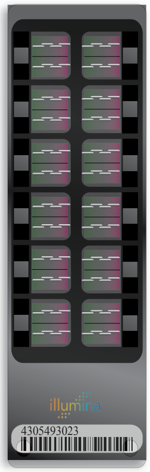
DNA methylation plays an important and dynamic role in regulating gene expression. It allows cells to become specialized and stably maintain those unique characteristics throughout the life of the organism, suppresses the deleterious expression of viral genes and other non-host DNA elements, and provides 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[1].

To enable cost-effective DNA methylation analysis for a variety of applications, Illumina offers a robust methylation profiling platform consisting of proven chemistries and the iScan and HiScan®SQ systems. The HumanMethylation450 BeadChip (Figure 1) offers a unique combination of comprehensive, expert-selected coverage and high throughput at a low price, making it ideal for screening large sample populations such as those used in genome-wide association study (GWAS) cohorts. By providing quantitative methylation measurement at the single-CpG–site level for normal and formalin-fixed parafin-embedded (FFPE) samples, 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.

Figure 1: Infinium HumanMethylation450 BeadChip



The Infinium HumanMethylation450 BeadChip features more than 450,000 methylation sites, within and outside of CpG islands.

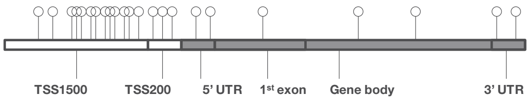
Importantly, coverage was targeted across gene regions with sites in the promoter region, 5'UTR, first exon, gene body, and 3'UTR in order to provide the broadest, most comprehensive view of methylation state possible (Figure 2). This multiple-site approach was extended to CpG islands/CpG island regions 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 the Illumina HumanMethylation27 BeadChip

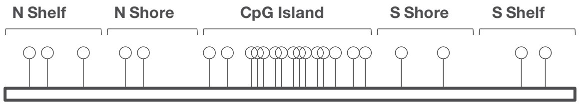
Streamlined Workflow

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

Figure 2: HumanMethylation450 BeadChip Provides Coverage Throughout Gene Regions



Feature Type	Genes Mapped	Percent Genes Covered	Number of Loci on Array
NM_TSS200	14895	0.79	2.56
NM_TS1500	17820	0.94	3.41
NM_5'UTR	13865	0.78	3.34
NM_1stExon	15127	0.80	1.62
NM_3'UTR	13042	0.72	1.02
NM_GeneBody	17071	0.97	8.97
NR_TSS200	1967	0.65	1.84
NR_TSS1500	2672	0.88	2.92
NR_GeneBody	2345	0.77	5.34



Feature Type	Islands Mapped	Percent Islands Covered	Average Number of Loci on Array
Island	26153	0.94	5.08
N_Shore	25770	0.93	2.74
S_Shore	25614	0.92	2.66
N_Shelf	23896	0.86	1.97
S_Shelf	23968	0.86	1.94

The HumanMethylation450 BeadChip offers broad coverage across gene regions, as well as CpG islands/CPG island regions, shelves, and shores for the most comprehensive view of methylation state.

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[2], 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 technologies (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>0.98), as well as with the HumanMethylation27 BeadChip and whole-genome bisulfite sequencing (Figure 4).

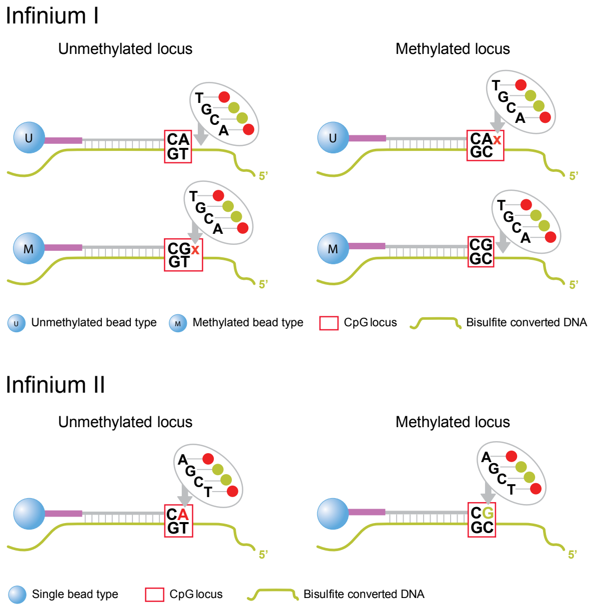
Sensitivity

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.

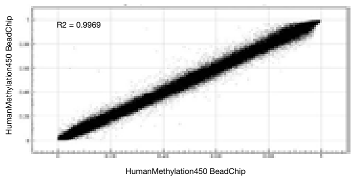
Figure 3: Broader Coverage Using Infinium I and II Assay Designs



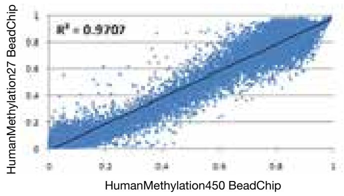
The HumanMethylation450 BeadChip employs both Infinium I and Infinium II assays, enhancing its breadth of coverage. Infinium I assay design employs two bead types per CpG locus, one each for the methylated and unmethylated states. The Infinium II design uses one bead type, with the methylated state determined at the single base extension step after hybridization.

Figure 4: High Assay Reproducibility

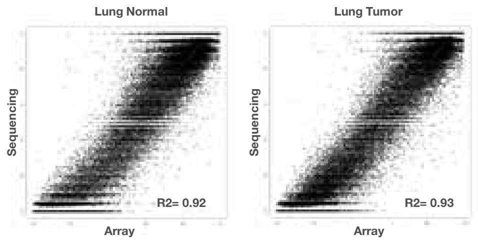
A: HumanMethylation450 Replicate Correlation



B: HumanMethylation27 vs. HumanMethylation450 Correlation

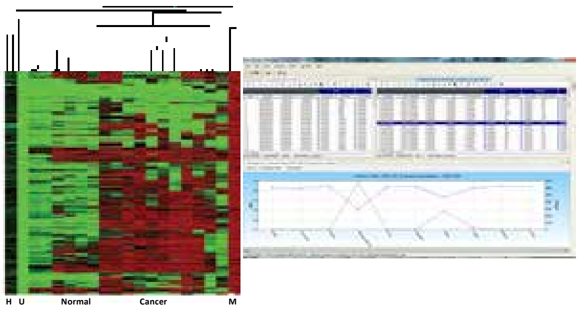


C. HumanMethylation450 vs. Whole-Genome Bisulfite Sequencing



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).

Figure 5: Integrated Data Analysis with Illumina GenomeStudio Software



GenomeStudio software supports DNA methylation analysis on any platform. Data are displayed in intuitive graphics. Gene expression data can be easily integrated with methylation projects (plotted on right).

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.

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.

Methylation Studies with FFPE Samples

Researchers can perform methylation studies on FFPE samples by using a special, modified version of the Infinium HumanMethylation450 BeadChip protocol[3] that leverages the easy-to-use Infinium FFPE DNA Restoration Solution[4,] to produce robust, highly reproducible results (Table 1). The FFPE DNA Restoration Solution includes the Illumina FFPE QC and the Infinium HD FFPE DNA Restore Kits. Please note that while the FFPE DNA Restoration Solution and HumanMethylation450 BeadChip kits are the same for normal and FFPE samples, investigators running FFPE samples should only follow the workflow described in the Infinium HD FFPE Methylation Assay protocol (manual or automated)[5,6], as it includes important changes to the standard protocols for each kit.

Table 1: Comparative Infinium HumanMethylation450 Data Quality Metrics—Standard vs. FFPE

HumanMethylation450 BeadChip	**Standard Protocol**	**FFPE Protocol**
Reproducibility (Technical replicates)	$r^2 \geq 98\%$	$r^2 \geq 98\%$
Number of sites detected*	≥ 99%	≥ 95%

*Based on non-cancer samples, recommended sample input amounts of high-quality DNA as confirmed by PicoGreen and following all other Illumina recommendations as per respective User Guides.

Ordering Information

Catalog No.	Product	Description
WG-314-1003	Infinium HumanMethylation450 BeadChip Kit (24 samples)	Each package contains two BeadChips and reagents for analyzing DNA methylation in 24 human DNA samples.
WG-314-1001	Infinium HumanMethylation450 BeadChip Kit (48 samples)	Each package contains four BeadChips and reagents for analyzing DNA methylation in 48 human DNA samples.
WG-314-1002	Infinium HumanMethylation450 BeadChip Kit (96 samples)	Each package contains eight BeadChips and reagents for analyzing DNA methylation in 96 human DNA samples.

Each HumanMethylation450 BeadChip can process 12 samples in parallel and assay >450,000 methylation sites per sample.

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 DNA methylation studies.

References

1. Portela A, Esteller M (2010) Epigenetic modifications and human disease. Nat Biotechnology 28: 1057–1068.
2. http://www.illumina.com/products/humanht_12_expression_beadchip_kits_v4.ilmn
3. Infinium HD FFPE DNA Restoration Protocol
4. http://www.illumina.com/products/infinium_ffpe_dna_restoration_solution.ilmn
5. Infinium HD FFPE Methylation Assay, Manual Protocol
6. Infinium HD FFPE Methylation Assay, Automated Protocol
7. Illumina FFPE QC Assay Protocol

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Pub. No. 270-2010-001 Current as of 09 March 2012