

Infinium® MethylationEPIC BeadChip

Affordable methylome analysis meets cutting edge content.

Infinium MethylationEPIC BeadChip Highlights

- Unique Combination of Coding Region and Enhancer-Wide Coverage, High-Throughput, and Low Cost Over 850,000 methylation sites per sample at single-nucleotide resolution
- High Assay Reproducibility
 > 98% reproducibility for technical replicates
- Simple Workflow
 PCR-free protocol with the powerful Infinium HD Assay
- Compatible 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 acquire and maintain a specialized state, suppresses the expression of viral and nonhost DNA elements, and facilitates 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, neurological disorders, aging, and development. 1, 2

To enable cost-effective DNA methylation analysis for various applications, Illumina offers a robust methylation profiling platform consisting of proven chemistries and the iScan® and HiScanSQ™ systems. The past 5 years of methylome research, including projects like ENCODE and FANTOM5, have identified enhancer regions as critical sites for differential methylation. The Infinium MethylationEPIC BeadChip (Figure 1) builds upon the industry-leading Infinium HumanMethylation450 BeadChip with > 90% of the original CpGs plus an additional 350,000 CpGs in enhancer regions. By providing quantitative methylation measurement at the single-CpG-site level for normal and formalin-fixed paraffin-embedded (FFPE) samples, this assay offers powerful resolution for understanding epigenetic changes.

Comprehensive Genome-Wide Coverage

The Infinium MethylationEPIC BeadChip provides unparalleled coverage of CpG islands, RefSeq genes, ENCODE open chromatin, ENCODE transcription factor binding sites, and FANTOM5 enhancers. Infinium HD technology enables content selection independent of bias-associated limitations often associated with methylated DNA capture methods.

Importantly, the MethylationEPIC BeadChip contains > 90% of the original Infinium Methylation450 BeadChip content, chosen to provide the broadest, most comprehensive view of methylation state possible (Figure 2).



Figure 1: Infinium MethylationEPIC BeadChip—The Infinium MethylationEPIC BeadChip features > 850,000 CpGs in enhancer regions, gene bodies, promoters, and CpG islands.

Also, the MethylationEPIC BeadChip targets regions identified by the ENCODE project as potential enhancers. ENCODE tracks for open chromatin, FAIRE assays, and transcription factor binding sites were overlaid to identify the most dynamic regions of the genome for content. Finally, the array targets enhancers identified by the FANTOM5 project across tissue types (Figure 3). The result is a pan-enhancer and coding region view of the methylome that can be used for epigenome-wide association studies on various human tissues and includes the following content categories requested by methylation experts:

- CpG sites outside of CpG islands
- Non-CpG methylated sites identified in human stem cells (CHH sites)
- Differentially methylated sites identified in tumor versus normal (multiple forms of cancer) and across several tissue types
- FANTOM5 enhancers
- ENCODE open chromatin and enhancers
- DNase hypersensitivity sites
- miRNA promoter regions
- > 90% of content contained on the Illumina HumanMethylation450 BeadChip

Streamlined Workflow

The MethylationEPIC BeadChip follows a user-friendly, streamlined workflow that does not require PCR. Its low sample input requirement (as low as 250 ng), enables analysis of valuable samples derived from limited DNA sources. MethylationEPIC BeadChip kits contain all required reagents for performing methylation analyses (except for the bisulfite conversion kit, which is available separately).

N Shelf	N Shore	CpG Island	S Shore	S Shelf
99 9				

Feature Type	# Covered	% Covered	Avg # Loci/Feature
Island	26,000	> 95%	6
North Shore	25,000	> 90%	3.5
South Shore	25,000	> 90%	3.5
North Shelf	22,000	> 80%	2
South Shelf	22,000	> 80%	2

Figure 2: MethylationEPIC BeadChip Provides Dense Coverage Throughout Gene Regions—The MethylationEPIC BeadChip offers broad coverage across gene regions, CpG islands/CpG island regions, shelves, and shores for the most comprehensive view of the methylation state. Specifications are rounded for product prerelease. Exact specifications will be released when product ships at the end of 2015.

High-Quality Data

The MethylationEPIC BeadChip applies both Infinium I and II assay chemistry technologies (Figure 4) 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. This enables each of up to 3 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 performance.

Precision and Accuracy

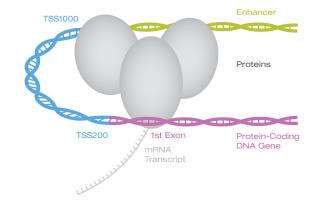
Reproducibility has been determined based on the correlation of results generated from technical replicates. The MethylationEPIC BeadChip showed strong correlation between replicates (r > 0.98), and with the HumanMethylation450 BeadChip. The Infinium HumanMethylation450 BeadChip showed a high R2 correlation with whole genome bisulfite sequencing (Figure 4).

Analytical Sensitivity

By comparing the results of replicate experiments (duplicates of 8 biological samples), Illumina scientists have shown that the Infinium Methylation BeadChips reliably detect a delta-beta value of 0.2 with a lower than 1% false positive rate.

Internal Quality Controls

Infinium HD-based assays produce high-quality data with sample-dependent and independent controls. The MethylationEPIC BeadChip includes negative controls to account for decreased sequence complexity after bisulfite conversion. The GenomeStudio® Methylation Module Software has an Integrated Controls Dashboard for easy monitoring of controls. For large-scale studies, the BeadArray Controls Reporter outputs a simple-to-scan excel file for rapid analysis of multiple controls.



Feature Type	# Features Mapped	% Features Covered	Avg # Loci/Feature
RefSeq			
NM_TSS200 ^a	> 20,000	> 88%	3
NM_TSS1500	> 23,000	> 97%	5
NM_5'UTR	> 20,000	> 85%	7
NM_1stExon	> 20,000	> 85%	2
NM_3'UTR	> 14,000	> 70%	1
NM_ExonBoundaries	> 8000	> 35%	0.5
NR_TSS200	> 4000	> 65%	1
NR_TSS1500	> 5000	> 80%	3
NR_ExonBoundaries	> 500	> 15%	0.2
GenCode Basic v12			
TSS200	> 65,000	> 86%	2
TSS1500	> 80,000	> 95%	5
5'UTR	> 50,000	> 75%	7
First Exon	> 45,000	> 60%	2
3'UTR	> 35,000	> 65%	3
Exon Boundaries	> 8000	> 30%	0.5
Enhancers			
ENCODE Open Chromatinb Evidence ≥ 4	> 150,000	> 65%	2
ENCODE TFBS in Open Chromatin ^c Evidence ≥ 3	> 220,000	> 50%	1
ENCODE TFBS in Open Chromatin Evidence ≥ 4	> 150,000	> 75%	3
FANTOM5 Enhancers ^d	> 23,000	> 80%	1

- a. Distance (in base pairs) from transcriptional start site (TSS).
- ENCODE Open Chromatin: Genomic regions ≥ 4 pieces of evidence supporting open chromatin status from ENCODE tracks within DNasel_FAIRE_ChIP_Synthesis_from_ ENCODE_OpenChrom_Duke_UNC_UTA.
- c. ENCODE TFBS in Open Chromatin: Regions of ENCODE Open Chromatin defined above also identified as transcription factor binding sites (TFBS) in the track TFBS_PeakSeqbased_Peaks. Data are provided with ≥ 3 and ≥ 4 pieces of evidence, as indicated.
 d. Genomic regions identified as enhancers by the FANTOM5 project.

Figure 3: MethylationEPIC BeadChip Provides Dense Coverage Throughout Enhancer Regions—The MethylationEPIC BeadChip offers dense coverage across multiple content categories requested by methylation experts including enhancer regions. Specifications are rounded for product prerelease. Exact specifications will be released when product ships at the end of 2015.

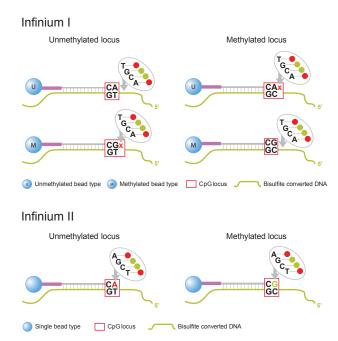


Figure 4: Broader Coverage Using Infinium I and II Assay Designs—
The MethylationEPIC BeadChip employs both Infinium I and Infinium II assays.
Infinium I assay design employs 2 bead types per CpG locus, 1 each for the methylated and unmethylated states. The Infinium II design uses 1 bead type, with the methylated state determined at the single base extension step after hybridization.

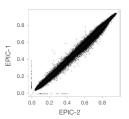
Integrated Analysis Software

MethylationEPIC BeadChip data analysis is supported by the GenomeStudio Methylation Module, enabling researchers to perform differential methylation analysis for small scale studies (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.

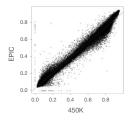
These tools and the GenomeStudio Genome Browser display valuable information such as chromosomal coordinates, percent GC, location in a CpG Island, and methylation β values. For large-scale studies, there are many freely available analysis packages that work in the software framework R for normalization and differential analysis of methylation data. $^{3.4}$ Instructional videos that explain the use of these data analysis packages are available on the Illumina website (www.illumina.com/multimedia/methylation-array-analysisvideos.ilmn).

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.

A. MethylationEPIC Replicate Correlation (R2 > 0.98)



B. HumanMethylation450 vs. MethylationEPIC Correlation (R2 > 0.98)



C. HumanMethylation450 vs. Whole Genome Bisulfite Sequencing

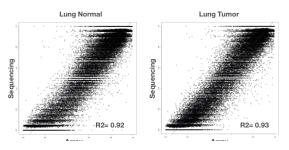


Figure 5: Infinium Methylation Technology Shows High Reproducibility and Correlation to Sequencing Data —A) Technical replicates of beta values for noncancer samples on the Infinium MethylationEPIC BeadArray show a > 98% R2 value. B) Comparison of beta values for noncancer samples on overlapping content between the Infinium HumanMethylation450 BeadArray vs the MethylationEPIC chip show an R2 of greater than 98%. C) Infinium HumanMethylation450K data show high methylation call correlation when compared to whole-genome bisulfite sequencing data across normal and tumor lung tissues.

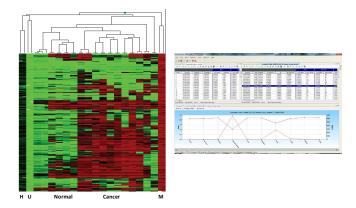


Figure 6: Integrated Data Analysis with Illumina GenomeStudio Software—GenomeStudio software supports DNA methylation analysis on any platform. Data are displayed in intuitive graphics (heat map, left). Gene expression data can be easily integrated with methylation projects (plotted on right).

Table 2: Ordering Information

Catalog No.	Product	Description
WG-317-1001	Infinium MethylationEPIC BeadChip Kit (16 Samples)	Each package contains 2 BeadChips and reagents for analyzing DNA methylation in 16 human DNA samples.
WG-317-1002	Infinium MethylationEPIC BeadChip Kit (32 Samples)	Each package contains 4 BeadChips and reagents for analyzing DNA methylation in 32 human DNA samples.
WG-317-1003	Infinium MethylationEPIC BeadChip Kit (96 Samples)	Each package contains 8 BeadChips and reagents for analyzing DNA methylation in 96 human DNA samples.
Each MethylationEF	PIC BeadChip can process 8 samples in parallel and assay > 850,000	methylation sites per sample.

Methylation Studies with FFPE Samples

Methylation studies on FFPE samples are enabled with a modified version of the MethylationEPIC BeadChip protocol. This protocol harnesses the Infinium FFPE DNA Restoration Solution to produce robust, reproducible results (Table 1). The Illumina FFPE QC and the Infinium HD FFPE DAN Restore Kits are included. THE FFPE DNA Restore Solution and MethylationEPIC 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), 7-9 as it includes important changes to the standard protocols for each kit.

Table 1: Comparative Infinium Methylation Data Quality Metrics-Standard vs. FFPE

Methylation BeadChip	Standard Protocol	FFPE Protocol
Reproducibility (Technical Replicates)	$r^2 \geq 98\%$	$r^2 \geq 98\%$
Number of sites detected*	≥ 96%	≥ 90%

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

Synergy with TruSeq Methyl Capture EPIC

In early 2016, Illumina will launch the TruSeq Methyl Capture EPIC panel, an enrichment-based bisulfite sequencing method that mirrors and expands upon the content of the MethylationEPIC chip. Now researchers can move seamlessly between array and sequencing technologies, taking advantage of the low price point on the MethylationEPIC chip for large-scale screens and diving deep on specific samples or subsets with the TruSeq Methyl Capture EPIC panel. These combined technologies represent the best of both worlds of methylation analysis. More details to come in 2016.

Summary

The MethylationEPIC BeadChip offers a unique combination of comprehensive, expert-selected coverage, high sample throughput capacity, and affordable price that makes it an ideal solution for large sample-size, genome-wide DNA methylation studies.

References

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- 5. Infinium HD FFPE DNA Restoration Protocol
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- 7. Infinium HD FFPE Methylation Assay, Manual Protocol
- 8. Infinium HD FFPE Methylation Assay, Automated Protocol
- 9. Illumina FFPC QC Assay Protocol

