DATA SHEET

Infinium™ MethylationEPIC v2.0 BeadChip

Genome-wide methylation screening with cutting-edge content

- Over 935K CpGs with expert-defined new content
- Highly accurate and precise DNA methylation data
- High-throughput analysis at minimal cost per sample
- Compatible with DNA extracted from FFPE tissue samples

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Introduction

DNA methylation plays an important role in regulating gene expression. Changes in cellular DNA methylation states have long been implicated in aging, development, and disease pathology.[1,2] For over a decade, Illumina has provided researchers with robust microarray-based tools powered by BeadArray™ technology to measure DNA methylation across the genome quantitatively. To date, the Infinium HumanMethylation450 and MethylationEPIC v1.0 BeadChips, have been used by the research community to drive data collection for epigenome-wide association studies (EWAS). These Infinium BeadChips have enabled both the discovery and application of methylation-based biomarkers in the fields of cancer research,[3,4] genetic diseases,[5] aging,[6] and molecular epidemiology.[7] The updated Infinium MethylationEPIC v2.0 BeadChip (Figure 1, Table 1), built upon the same reliable Infinium chemistry foundation, offers enhanced, expert-selected content, to enable more biological discoveries in a new era of epigenetics research.

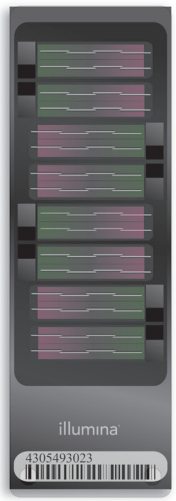

Figure 1: The Infinium MethylationEPIC v2.0 BeadChip—The Infinium MethylationEPIC v2.0 BeadChip features > 935,000 CpGs in enhancer regions, gene bodies, promoters, and CpG islands.

Table 1: Product information

Feature	Description
Species	Human
Total no. of markers[a]	> 935,000
No. of samples per BeadChip	8
DNA input requirement	250 ng
Specialized sample types	FFPE tissue
Assay chemistry	Infinium HD
Instrument support	iScan, NextSeq 550 systems

a. Methylation sites interrogated.

Genome-wide, cutting-edge content

The Infinium MethylationEPIC v2.0 BeadChip builds upon the genome-wide backbone of the Infinium MethylationEPIC v1.0 BeadChip, maintaining high backwards compatibility (Figure 2), while adding new content informed by expert feedback and epigenetic evaluations of human cancers and cellular samples (Table 2, Table 3). Nonfunctional probes routinely filtered out in DNA methylation studies due to underlying single nucleotide polymorphisms (SNPs), cross-hybridization, and multimapping behavior[8] were removed in the new version of the BeadChip, leaving space for more functional content identified by the epigenetics community.

Over 186,000 new probes were designed to target known enhancers, super-enhancers, CTCF-binding domains, and open regions of chromatin associated with primary tumors identified by Assay for Transposable-Accessible Chromatin using sequencing (ATAC-Seq) and chromatin immunoprecipitation sequencing (ChIP-Seq) experiments. This new content was advised by leading epigenetic researchers and recent scientific publications.[9-14]

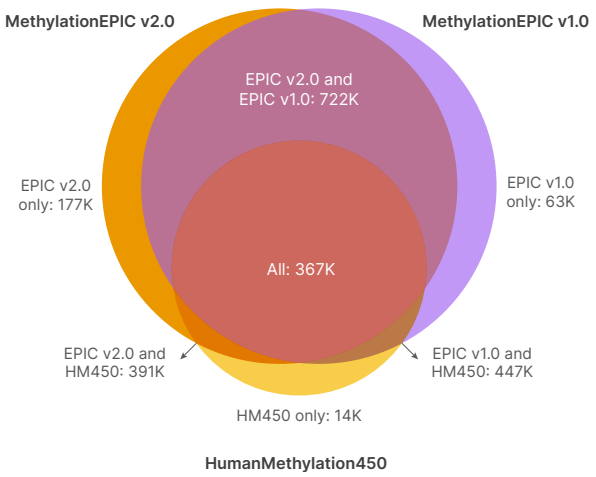

Figure 2: High backwards compatibility with previous BeadChips—The Infinium MethylationEPIC v2.0 BeadChip builds upon the existing CpG backbones of the Infinium MethylationEPIC v1.0 and HumanMethylation450 BeadChips.

As another improvement, CpG islands and exons that were covered insufficiently on the Infinium MethylationEPIC v1.0 BeadChip are covered thoroughly with additional probes. Additionally, > 450 cancer driver mutations are queried in the new version, making the Infinium MethylationEPIC v2.0 BeadChip a multiomics tool for cancer studies.[15] However, researchers in all areas of study can take advantage of the new cutting-edge content for their future epigenetic discoveries.

Table 2: Dense coverage of CpG islands

Feature	No. covered	% covered	Avg no. of loci/feature
Island	25,381	91%	5.4
North shore	25,115	90%	3.5
South shore	24,870	89%	3.6
North shelf	21,719	78%	2.1
South shelf	21,677	78%	2.1

Legacy content in the Infinium MethylationEPIC v1.0 and v2.0 BeadChips:

- CpG islands
- Non-CpG (CHH) methylated sites identified in human stem cells sites
- ENCODE open chromatin and enhancers
- FANTOM5 enhancers
- DNase hypersensitivity sites
- miRNA promoter regions
- > 85% HumanMethylation450 BeadChip content

New content in the Infinium MethylationEPIC v2.0 BeadChip includes:

- Differentially methylated sites identified in tumor versus normal samples for multiple cancers
- Enhancers and super-enhancers identified by ChIP-Seq in cancer and cell line samples
- Differentially accessible chromatin regions identified in primary human cancers using ATAC-Seq
- Expanded coverage of CpG islands
- Enhanced exon coverage for more accurate copy number variation (CNV) detection
- Common cancer driver mutations

Table 3: Infinium MethylationEPIC v2.0 coverage of genomic regions

Feature type	No. of features mapped	% features covered	Avg no. of loci/ feature
RefSeq			
NM_TSS200[a]	51,688	82%	2.8
NM_TSS1500	59,981	96%	5.6
NM_5′ UTR	42,051	67%	1.7
NM_1stExon	44,471	71%	1.8
NM_3′ UTR	39,407	63%	1.3
NM_Exonic	207,398	28%	0.5
NR_TSS200	12,706	68%	2.0
NR_TSS1500	15,961	86%	3.9
NR_1stExon	9810	53%	1.4
NR_Exonic	30,211	25%	0.5
GenCode Basic v41			
TSS200	160,572	79%	1.7
TSS1500	197,603	80%	3.9
5' UTR	61,823	59%	1.4
First Exon	118,516	47%	1.1
3' UTR	41,659	53%	1.2
Exonic	417,055	26%	0.5
Enhancers			
DNase hypersensitivity sites[b]	432,393	16%	0.2
FANTOM5 Enhancers[c]	23,852	84%	1.0
CisReg Site Evid 40-50[d]	19,159	70%	1.3
CisReg Site Evid 50-60	21,609	67%	1.2
CisReg Site Evid 60-70	30,152	61%	1.1
CisReg Site Evid 70-80	66,446	47%	0.8
CisReg Site Evid > 80	153,712	19%	0.3
Cancer driver mutations			
Cancer driver mutations[e]	473	81%	0.8

a. Distance in base pairs from transcriptional start site (TSS).
b. From ENCODE v5: 2745580 DNase hypersensitivity sites genome-wide.
c. Genomic regions identified as enhancers by the FANTOM5 project.
d. From ENCODE v5: 87 studies with full data annotation.
e. From: Bailey MH, Tokheim C, Porta-Pardo E, et al. Comprehensive Characterization of Cancer Driver Genes and Mutations. Cell. 2018;173(2):371-385.e18.

Streamlined workflow

The Infinium MethylationEPIC v2.0 BeadChip Kit follows a straightforward, user-friendly workflow that does not require sample pooling and indexing. The 8-sample BeadChip is processed following the Infinium HD Methylation Assay and scanned on the iScan™ or NextSeq™ 550 systems.

The Infinium methylation assay has been optimized with new rapid bisulfite conversion methods, reducing the overall assay time (DNA extraction to intensity files) from four days to three. Bisulfite conversion, completed before the Infinium methylation assay, can now be completed in three hours with recommended third-party rapid bisulfite conversion kits. These kits also support automation, reducing variability while increasing sample processing throughput.

The Infinium MethylationEPIC v2.0 BeadChip kits contain all required reagents for performing methylation screening, except for the bisulfite conversion kits, which are purchased separately.

Learn more by reading the Automated bisulfite conversion for Infinium Methylation BeadChips technical note.

Highly accurate and precise methylation data

Infinium array chemistry employs many bead replicates for each CpG site queried, each with thousands of probes attached to it. As a result, the Infinium methylation assay provides highly precise methylation measurements. This is evidenced by internal experiments with cancer cell lines which show > 99% reproducibility between technical replicates (Figure 3A). Additionally, high analytical sensitivity is achieved with the Infinium methylation assay being able to detect differences in beta values of 0.2 with a lower than 1% false positive rate. Experiments also show high correlation between overlapping assays from Infinium MethylationEPIC v2.0 and v1.0 BeadChips (Figure 3B) and methylation sequencing data (Figure 3C). Studies have shown that the level of accuracy and precision obtained with Infinium BeadChips can only be obtained with a high sequencing depth of 100× or greater.[16]

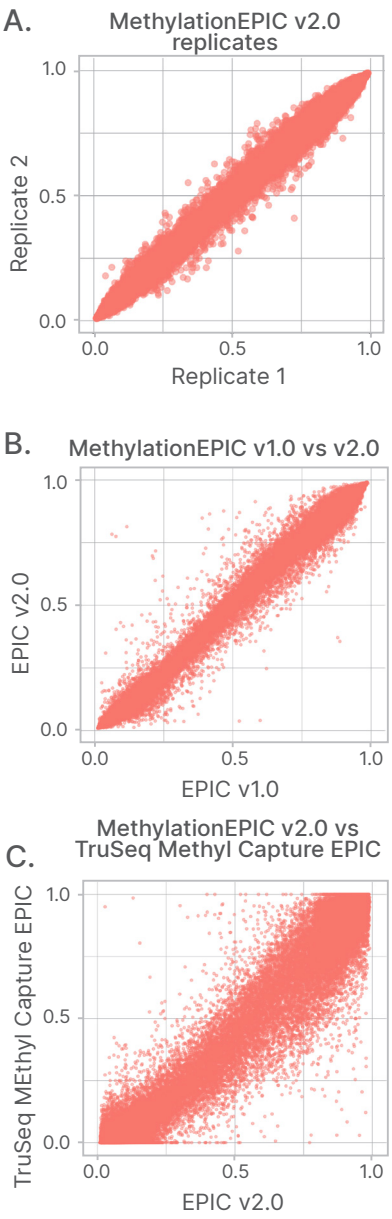

Figure 3: Infinium MethylationEPIC v2.0 BeadChips show high reproducibility, backwards compatibility, and correlation to sequencing data—(A) Comparison of beta values from technical replicates for HeLa samples on MethylationEPIC v2.0 show a > 99% R^2 value. (B) Comparison of beta values for HeLa samples on overlapping content between MethylationEPIC v1.0 and v2.0 show a > 99% R^2 value. (C) MethylationEPIC v2.0 data show high methylation call correlation (R^2 > 96%) when compared to targeted bisulfite sequencing data at 100× sequencing depth. Concordance plots were developed using the beta values generated from the SeSAMe data analysis package.

Straightforward, integrated QC

Infinium methylation assays contain sample-dependent and independent controls for simple and intuitive quality control (QC). These controls indicate the quality of data as a function of the Infinium workflow steps, and sample-specific controls such as bisulfite conversion efficiency and negative controls. Controls can be assessed in a tabulated format using the BeadArray Controls Reporter, or visually across multiple samples using the GenomeStudio™ Methylation Module Software. In addition, the user base has devised additional methods to assess data quality using third-party software solutions such as SeSAMe[17] and minfi.[18]

Simple secondary data analysis

The widespread use of Infinium methylation BeadChips for over a decade has led to the development of user-friendly R packages for data analysis by the user community. These packages, such as SeSAMe and minfi, provide the most updated bioinformatics methods for normalization, probe filtering, and detection of differential methylation. Instructional videos and comprehensive user guides explain the use of these data analysis packages. In addition, due to the targeted nature of BeadArray technology, the output data can be easily processed with minimal computing power and stored at little to no cost.

FFPE sample compatibility

Formalin-fixed, paraffin-embedded (FFPE) tissue samples show robust performance with a modified version of the Infinium Methylation HD Assay (Table 4). For precious samples and optimal sample integrity the Infinium FFPE QC and DNA Restoration Kits are recommended.

Table 4: Robust data with FFPE samples

Infinium MethylationEPIC BeadChips	Standard	FFPE
Reproducibility (technical replicates)	r^2 ≥ 98%	r^2 ≥ 98%
No. of sites detected[a]	≥ 96%	≥ 90%

a. 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.

Summary

The Infinium MethylationEPIC v2.0 BeadChip offers an accessible genome-wide methylation analysis tool with cutting-edge content and high throughput functionality, making it an ideal solution for epigenetics studies of all sizes.

Learn more

Infinium MethylationEPIC v2.0 BeadChip, illumina.com/products/by-type/microarray-kits/infinium-methylation-epic.html

Infinium MethylationEPIC v2.0 BeadChip support support.illumina.com/array/array_kits/infinium-methylationepic-beadchip-kit.html

Methylation Array Analysis, illumina.com/techniques/microarrays/methylation-arrays.html

Methylation Array Data Analysis Tips, illumina.com/techniques/microarrays/methylation-arrays/methylation-array-data-analysis-tips.html

Rapid, Automated Bisulfite Conversion, illumina.com/content/dam/illumina/gcs/assembled-assets/marketing-literature/automated-bisulfite-infinium-methylation-tech-note-m-gl-00144/automated-bisulfite-Infinium-methylation-tech-note-m-gl-00144.pdf

Cancer Epigenetics, illumina.com/areas-of-interest/cancer/research/cancer-epigenetics.html

Ordering information

Product	Catalog no.
Infinium MethylationEPIC v2.0 BeadChip Kit (8 samples)	20087706
Infinium MethylationEPIC v2.0 BeadChip Kit (16 samples)	20087707
Infinium MethylationEPIC v2.0 BeadChip Kit (32 samples)	20087708
Infinium MethylationEPIC v2.0 BeadChip Kit (96 samples)	20087709

Each Infinium MethylationEPIC v2.0 BeadChip can process eight samples in parallel and assay > 935,000 methylation sites per sample.

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