

Infinium™ Mouse Methylation BeadChip

Features > 275K markers across the methylome for high-resolution epigenetic analyses of diverse murine strains.

Highlights

- Comprehensive coverage of coding region and enhancer-wide content**
 Interrogates > 275K methylation sites per sample at single-nucleotide resolution
- Simple workflow is compatible with FFPE samples**
 Provides PCR-free protocol using the powerful Infinium HD assay for epigenetic studies
- High assay reproducibility**
 Achieves > 98% reproducibility for technical replicates
- Broad range of applications**
 Enables epigenome-wide association studies, xenograft experiments, preclinical research, and more

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 normal and aberrant biological 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™ System. The last decade of methylome research, using the Infinium Human Methylation450 and Infinium MethylationEPIC BeadChips, has yielded foundational information for understanding human biology and disease mechanisms. Conversely, researchers have lacked methylation array tools for the analysis of model organisms used to simulate disease states in human beings.

To address this need, Illumina offers the Infinium Mouse Methylation BeadChip (Figure 1). With content developed through a collaboration between Life Epigenetics and Drs. Peter Laird, Hui Shen, and Wanding Zhou of the Van Andel Institute, the Infinium Mouse Methylation BeadChip enables epigenetic analysis of virtually all murine strains used in research laboratories, including wildtype, knockout, transgenic, and other types of engineered mice. By providing quantitative methylation measurement at the single-CpG-site level for normal and formalin-fixed paraffin-embedded (FFPE) samples, this array offers powerful resolution for understanding epigenetic changes.

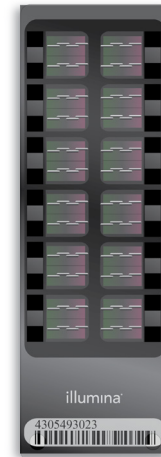


Figure 1: Infinium Mouse Methylation BeadChip—The Infinium Mouse Methylation BeadChip features > 275K CpGs in enhancer regions, gene bodies, promoters, CpG islands, and other regions of the methylome.

Comprehensive genome-wide coverage

The Infinium Mouse Methylation BeadChip provides balanced coverage of CpG islands, transcription start sites, enhancers, imprinted loci, gene body regions, repetitive element regions, lamin attachment domains, CTCF binding sites, and hypermethylated regions in cancer (Table 1 and Figure 2). Infinium HD chemistry enables content selection independent of bias-associated limitations often associated with methylated DNA capture methods.

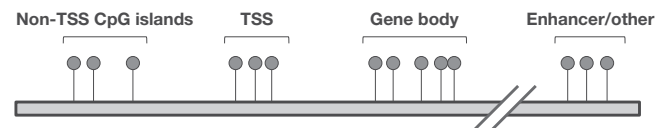


Figure 2: Dense coverage throughout gene regions—The Infinium Mouse Methylation BeadChip offers broad coverage across gene regions, CpG islands/CpG island regions, transcription start site (TSS), and other regions for a comprehensive view of the methylation landscape in the murine genome.

Streamlined workflow

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

Table 1: Summary of coverage

Feature	Description of coverage	Total no. of probes
Transcription start sites (TSS), within 500 bp of protein-coding transcripts	Covers 28,847 protein-coding transcripts	> 88,000
miRNA promoter TSS	Covers all annotated miRNA from Ensembl v75; 1,972 miRNAs total	> 1600
Noncoding RNA TSS	Covers lncRNA sites as annotated by Ensembl v75	> 4700
Enhancer	Sites defined by VISTA and mouse ENCODE; 63,307 enhancers total	> 133,000
Imprinted loci	Sites selected by whole-genome bisulfite sequencing (WGBS) where intermediate methylation is observed across various cell types.	> 800
CpG island	Selected from University of California, Santa Cruz (UCSC) mouse CpG island collection where TSS does not overlap with CpG island; 16,023 CpG islands total	> 32,000
Hypermethylated regions in cancer	Selected from WGBS data of mouse tumors and normal tissue; 5756 CpG sites total	> 5700
Non-CpG cytosine probes (CpH sites)	CpH sites evenly distributed by sequence context to CAG, CTG, CCG, CAH, CTH, and CCH sites; ~4000 sites total	> 13,500
Orphan CpG probes in lamin attachment domains	Selected from mapping predictions of human mega-base scale partially methylated domains onto the mouse genome	> 11,400
CpGs from consensus repetitive elements	Selected for conserved sequences for Line1, B1, and IAPEY1 elements	70
Gene body sites	Random sites > 2 kb from protein TSS	> 143,000
CTCF binding sites	Selected from sequences that overlap CTCF ChIP-Seq peaks from Mouse ENCODE	> 10,000
Human MethylationEPIC liftover	Selected from the most highly conserved Human MethylationEPIC probes mapped onto the mouse genome	> 25,000
Random CpG probes	Sites randomly selected to reduce bias in the assessment of global genomic DNA methylation	3000
Strain-specific SNP probes	One from each of the 20 chromosomes for the 18 mouse strains covered by the mouse genome project	358

Robust assay chemistry

The Infinium Mouse Methylation BeadChip applies both Infinium I and II assay chemistries (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. This enables 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.

Reproducibility

Reproducibility has been determined based on the correlation of results generated from technical replicates. The Infinium Mouse Methylation BeadChip shows strong correlation between replicates ($r^2 > 0.98$) (Figure 4A). The Infinium Mouse Methylation BeadChip also shows a high r^2 correlation with whole genome bisulfite sequencing data (Figure 4B).

Analytical sensitivity

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

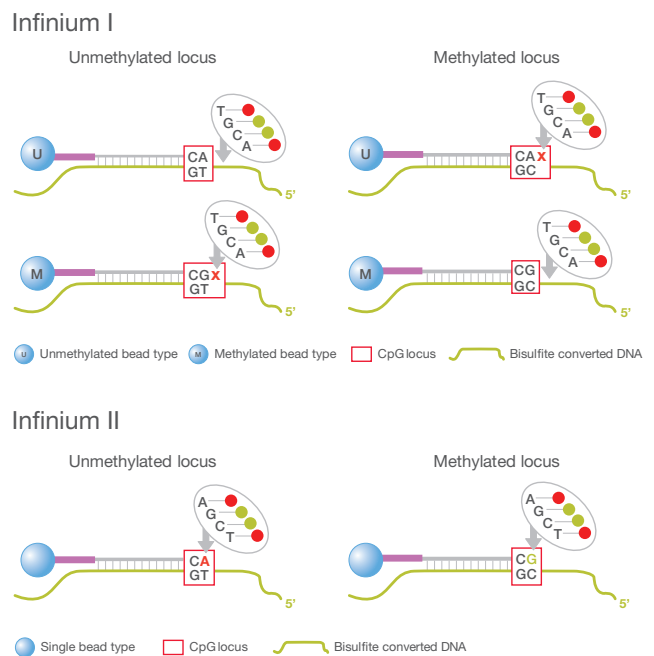


Figure 3: Broad coverage using Infinium I and II assay designs—The Infinium Mouse Methylation BeadChip employs both Infinium I and Infinium II assays. Infinium I assay design employs two bead types per CpG locus, one each for the methylated and unmethylated states. The Infinium II design uses one bead type, with the methylated state determined at the single base extension step after hybridization.

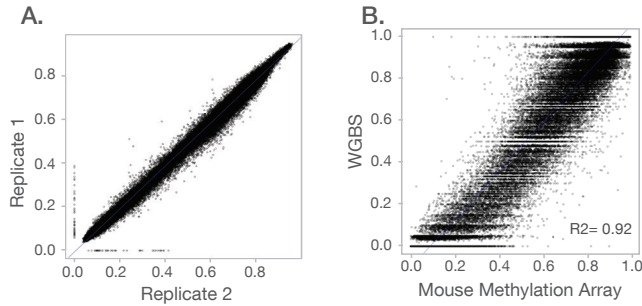


Figure 4: Infinium methylation technology shows high reproducibility and correlation to sequencing data—(A) Technical replicates of beta values for NIH3T3 DNA samples on the Infinium Mouse Methylation BeadChip show a $> x\%$ r^2 value. (B) Infinium Mouse Methylation BeadChip data show high methylation call correlation when compared to WGBS data across NIH3T3 cells.

Internal quality controls

Infinium HD assays produce high-quality data with sample-dependent and -independent controls. The Infinium Mouse Methylation 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.

Integrated analysis software

Infinium Mouse Methylation BeadChip data analysis is supported by the GenomeStudio Methylation Module, enabling researchers to perform differential methylation analysis for small-scale studies. GenomeStudio Software features advanced visualization tools that enable researchers to view vast amounts of data in a single graph, such as heat maps and other plots (Figure 5).

High compatibility with other software

Third-party solutions can use the same intensity files from the Infinium Mouse Methylation BeadChip run on the iScan System. 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}

Methylation studies with FFPE samples

Methylation studies on FFPE samples are enabled with a modified version of the Infinium Mouse Methylation BeadChip protocol. This protocol harnesses the Infinium FFPE DNA Restoration Kit to produce robust, reproducible results. The Illumina FFPE QC and the Infinium HD FFPE DNA Restore Kits are supported. Investigators running FFPE samples should only follow the workflow described in the Infinium HD FFPE Methylation Assay protocol (manual or automated), as it includes important information for the standard protocols for each kit.⁵⁻⁸

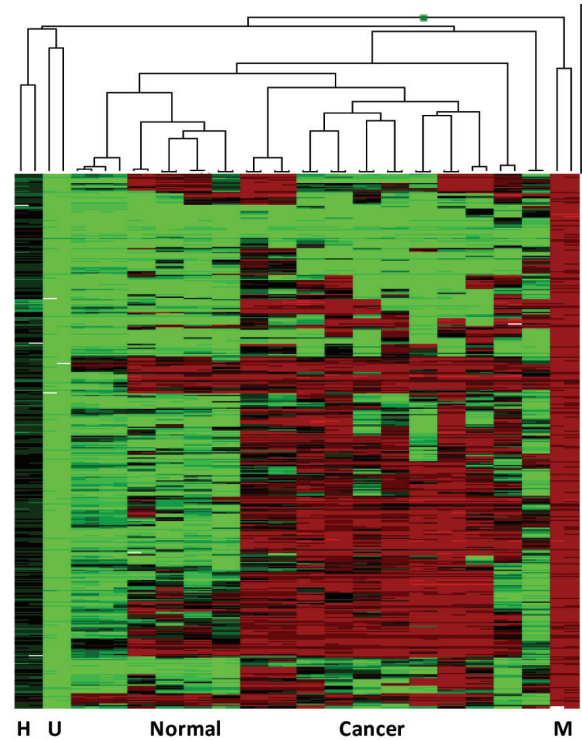


Figure 5: Integrated data analysis with Illumina GenomeStudio Software—GenomeStudio Software supports DNA methylation analysis on any platform. Data are displayed in intuitive graphics (heatmap).

Broad range of applications

The Infinium Mouse Methylation BeadChip enables a broad range of applications from basic science research to preclinical studies. There are over 200K modified mouse strains, many of which were created to model human disease. The Infinium Mouse Methylation BeadChip allows for the comprehensive methylome analysis of mice across multiple tissues, allowing for the analysis of genetic regulation in target tissues of specific disease models. The Infinium Mouse Methylation BeadChip, with a design that accommodates unmodified mouse strains as well, can be used for epigenome-wide association studies. These studies are facilitated in mice due to the ability to carry out specific tests for DNA methylation changes caused by environmental exposures in specific tissues for subjects with a uniform genetic background.

Additionally, the Infinium Mouse Methylation BeadChip can be used with patient-derived xenograft models of cancer for analysis of stromal features of cancer, which may play a role in human tumor growth and progression. The array can also be used in preclinical research to test whether experimental therapeutics have the intended effects in target tissues. Genetic toxicologists potentially could find applications for the Infinium Mouse Methylation BeadChip to understand the mechanisms of toxicity in the context of drug or environmental pollutant research. Developmental biologists might also use the array to investigate the role of DNA methylation in different tissues during embryonic development. Beyond these examples, the Infinium Mouse Methylation BeadChip potentially can be used in any application where a researcher wants to leverage DNA methylation as a readout for genetic regulation in the mouse genome.

Summary

The Infinium Mouse Methylation BeadChip offers a unique combination of comprehensive, expert-selected coverage and high sample throughput that makes it an ideal solution for genome-wide DNA methylation studies with a large number of samples.

Learn more

Learn more about the Infinium Mouse Methylation BeadChip and other Illumina genotyping products and services at www.illumina.com/techniques/microarrays.html.

References

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2. Rakyan VK, Down TA, Balding DJ, Beck S. *Epigenome-wide association studies for common human diseases*. *Nat Rev Genetics*. 2011;12(8):529–541.
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4. Assenov Y, Muller F, Lutsik P, et al. *Comprehensive analysis of DNA methylation with RnBeads*. *Nat Methods*. 2015;11(11):1138–1140.
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Ordering Information

Infinium Mouse Methylation BeadChip Kit	Description	Catalog no.
24 samples	2 BeadChips and reagents for analyzing DNA methylation in 24 mouse DNA samples.	20041558
48 samples	4 BeadChips and reagents for analyzing DNA methylation in 48 mouse DNA samples.	20041559
96 samples	8 BeadChips and reagents for analyzing DNA methylation in 96 mouse DNA samples.	20041560

Each Infinium Mouse Methylation BeadChip can process 12 samples in parallel and assay > 275K methylation sites per sample.