APPLICATION NOTE

Use of the Infinium™ Mouse Methylation BeadChip for epigenetic analysis

Accurate, repeatable methylation quantification for mouse studies

- Quantify DNA methylation across the mouse genome
- Benchmark and validate engineered mouse models of human disease
- Analyze the murine contribution to DNA methylation in patient-derived xenograft models
- Quantify epigenetic changes due to exogenous treatments and chemical exposures

illumina®

Introduction

DNA methylation is an epigenetic modification that plays an instrumental role in gene regulation.[1] Unlike genetic sequence information, DNA methylation is a dynamic biomarker that is responsive to environmental stimuli. For this reason, it can be used to gain insights into environmental exposures and disease states.[2]

While somatic cells share genetic information, methylation can differ between cells based on temporal and spatial cues. These epigenetic differences underlie and contribute to early development and cell and tissue differentiation.[3] Methylation changes are also observed in cancer, a class of diseases characterized by aberrant tissue growth and gene expression.[4,5]

Because methylation is an impermanent DNA modification and varies between tissue types and states, much remains unknown about functional implications of differential DNA methylation. Recent research has begun to establish specific methylation patterns as biomarkers with applications in early cancer detection and classification of tumor types and subtypes.[4,5] These studies indicate that DNA methylation is able to serve as an important tool for the detection and classification of human disease.

Two main methods are currently available for assessing the methylation status of DNA, bisulfite sequencing and methylation microarrays. Bisulfite sequencing has the capability to detect the majority of methylated cytosine bases in the genome, or a targeted subset of the genome, while microarrays have probes targeted to specific sites.

In a published comparison of both methods, Illumina methylation arrays and targeted bisulfite sequencing both performed similarly in terms of coverage of genome-wide elements. However, methylation arrays were determined to be superior in terms of precise quantification of DNA methylation.[6] Illumina Infinium Methylation Arrays also offer a simplified bioinformatic workflow and cost advantages when compared to sequencing approaches.

One limitation of arrays is that the current offerings do not include tools for common model organisms in biomedical research, including murine species. There are over 200,000 engineered mice commercially available through entities such as the Jackson Laboratory; however, researchers working with these mice have not had cost-effective array tools for their epigenetic studies.

The Infinium Mouse Methylation BeadChip offers comprehensive coverage of the mouse methylome, targeting 285K CpG sites that include CpG islands, transcription start sites, enhancers, imprinted loci, gene body regions, repetitive element regions, lamin attachment domains, CCCTC-binding factor (CTCF) binding sites, and hypermethylated regions observed in cancer. The BeadChip is highly flexible and can be used with all murine strains and genetically engineered mouse models. It can also be used in patient-derived xenograft (PDX) models of cancer for analysis of stromal features. Studies with genetically identical mice allow researchers to control for genetic variation and different environmental exposures, simplifying biomarker discovery in epigenome-wide association studies.

This application note presents selected data to demonstrate the ability of the Mouse Methylation BeadChip to accurately quantify methylation across the genome, differentiate between tissue-type samples, and analyze PDX samples. Functionality of this array was evaluated using experiments performed at Illumina and the Van Andel Research Institute. The complete study, *DNA Methylation Dynamics and Dysregulation Delineated by High-Throughput Profiling in the Mouse*, is available online.[7]

Methods

For this study, 120 murine samples were analyzed at the Van Andel Research Institute and at Illumina in San Diego, CA. Mouse methylated genomic DNA controls for methylation titration experiments were purchased from EpigenDx Inc. (Catalog no. 80-8060M-PreMix). Samples were processed following the standard Illumina Infinium BeadChip workflow using Zymo Classic Bisulfite Conversion kits (Catalog no. D5001) to resolve cytosine from 5-methylcytosine. Bisulfite-converted samples processed with the Infinium workflow were loaded on Infinium Mouse Methylation BeadChips and run on the iScan™ System.

Quality control metrics were analyzed using GenomeStudio™ software and the third-party SeSAMe software package.[8,9] For data analysis, the SeSAMe

pipeline, OpenSeSAMe, was used. Probes with a detection p-value > 0.05 across all passing samples were not carried forward into the analysis.

Results

Methylation quantification

To test the ability of the Infinium Mouse Methylation BeadChip to resolve changes in DNA methylation within experimental samples, commercially available methylated and unmethylated mouse DNA standards were mixed in discrete ratios from 0% to 100% and assayed on the BeadChip. β-values ranging from 0 to 1 indicate the proportion of unmethylated to methylated DNA, with 0 being fully unmethylated and 1 being fully methylated. As expected, β-values for the methylation standards increased corresponding to the proportion of DNA methylation of input sample. Test results were reproduced at two separate laboratories, demonstrating that similar results are independently achieved by following the Infinium Mouse Methylation BeadChip protocol (Figure 1).[10]

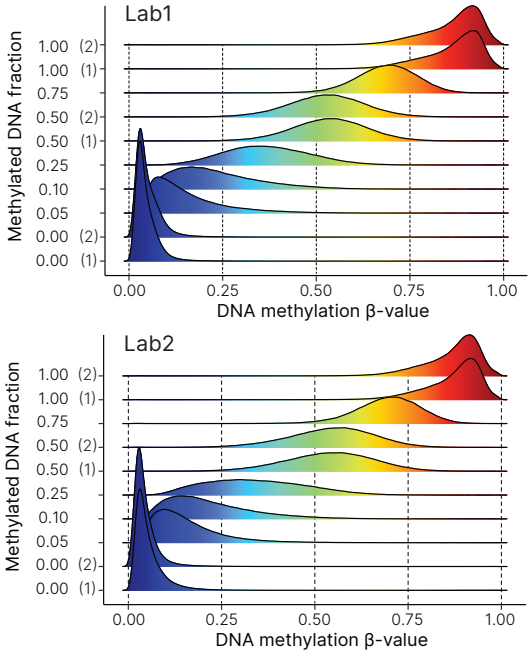

Figure 1: Accuracy of Infinium Mouse Methylation BeadChip DNA methylation quantification—β-values indicating the fraction of methylated DNA in titrated DNA standards, analyzed independently at two sites. Y-axis values in parentheses indicate experimental replicates.

Input sensitivity

Next, the impact of the amount of input DNA on the number of probes passing detection was measured using the OpenSeSAMe detection cutoff. Illumina recommends 250 ng for the minimum input amount of DNA. With 250 ng DNA input, > 90% of probes pass the OpenSeSAMe detection cutoff. However, the results also show that usable data can be obtained even when using 5 ng of DNA as input (Figure 2). Below 250 ng, the probes passing the cutoff decreases incrementally in relation to DNA input amount to approximately 60% at 5 ng (data not shown). Lowered input also results in the decrease of measurements of intermediate β-values, whereas measurements of more extreme values are relatively unaffected.

Experimental reproducibility

Additional tests were performed to assess reproducibility of the Infinium Mouse Methylation BeadChip using mouse tumor-derived DNA. In these tests, four different mouse colon tumor types were run in eight-fold replicates and processed at two separate laboratories. Samples were run on five BeadChips and randomized in terms of array position and BeadChip. The heat map shows that DNA methylation profiles were distinct across different tumor

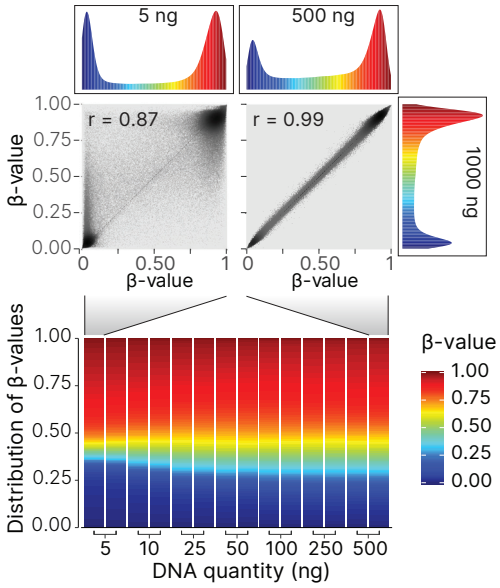

Figure 2: Methylation quantification for low DNA sample inputs—Pearson r correlations are shown for 5 ng and 500 ng sample input compared to 1000 ng. Observed r^2 correlation values decrease as the input level is changed from 500 ng to 5 ng. Decreasing the DNA input below 250 ng results in an increasing number of probes that do not pass the detection cutoff, and intermediate β-values decrease, especially when sample input is reduced below 100 ng.

types, but highly consistent within sample replicates, regardless of processing lab, assay plate, or BeadChip (Figure 3).

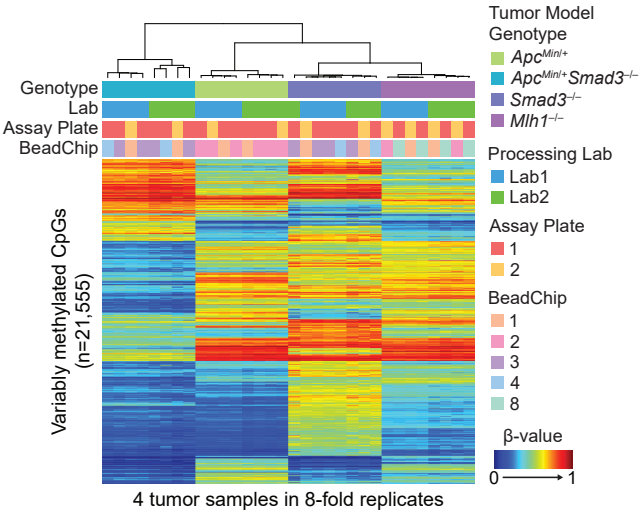

Figure 3: Experimental reproducibility of the Infinium Mouse Methylation BeadChip—β-value heat map shows clustering of 32 samples from four mouse model tumor types. Data were generated on five separate Infinium Mouse Methylation BeadChips run at two different sites.

Unsupervised clustering analysis carried out on the 21,555 most variably methylated probes across the data set showed that the Mouse Methylation BeadChip provided reproducible data independent of processing lab or BeadChip position variables (data not shown). The Pearson correlation coefficient for technical replicates on the same plate and BeadChip position was 0.9924 (n=16 pairs) for both processing labs. The mean correlation coefficient for technical replicates from the same plate at different BeadChip positions was 0.9945 (n=22 pairs). Finally, the mean correlation coefficient from replicate samples run at the same lab, from different plates, at the same BeadChip position was 0.9981 (n=2 pairs). These results show that the Infinium Mouse Methylation BeadChip provides reproducible results with minimal impact of BeadChip, sample position within a BeadChip, or processing lab.

Degraded sample performance

To test how well the Infinium Mouse Methylation BeadChip would perform with degraded DNA, adjacent mouse tissue was prepared by using a fresh freezing, or formalin-fixed paraffin-embedded (FFPE) process at 24 hr and 48 hr. The DNA samples from the FFPE samples were prepared using the Infinium HD FFPE DNA Restore Kit (Catalog no. WG-321-1002) and run following the Infinium Mouse Methylation BeadChip protocol. For comparison, the fresh frozen samples were prepared following the standard protocol. The correlation between methylation measurements for the fresh frozen tissue and FFPE samples was 0.9875, demonstrating that the Infinium Mouse Methylation BeadChip can be used with samples with DNA samples purified from FFPE tissue sources (Figure 4).

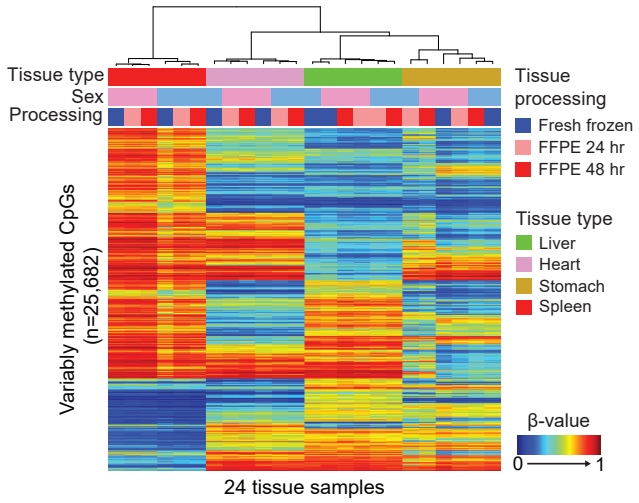

Figure 4: Performance of the Infinium Mouse Methylation BeadChip with FFP-damaged DNA—Adjacent samples from four tissue types were prepared by fresh freezing, or FFPE fixation at 24 and 48 hr. Methylation quantification between FFPE and frozen samples showed minimal differences (Pearson's r = 0.9875).

Patient-derived xenograft differentiation

DNA samples derived from PDX models contain mixtures of human and mouse DNA and researchers may want to analyze the murine component using the Infinium Mouse Methylation BeadChip. To determine the effectiveness of Infinium arrays in PDX applications, probe sets were identified as predicted to work for both species, or specific to only one species, on the human Infinium MethylationEPIC BeadChip and Infinium Mouse Methylation BeadChip. Both arrays were tested with titrated mixes of bisulfite-treated mouse and human DNA. Other than probes predicted to function with mouse and human DNA, no mouse-specific probes passed detection when 100% human DNA

was applied to the Infinium Mouse Methylation BeadChip (Figure 5A). When increasing the ratio of mouse-to-human DNA, more mouse-specific probes pass the detection cutoff. Similarly, when using Infinium MethylationEPIC BeadChip with mouse DNA samples, the only probes that pass the detection cutoff were probes predicted to function in both species (Figure 5B). Increasing the ratio of human-to-mouse DNA applied to the Infinium MethylationEPIC BeadChip results in more human-specific probes passing the detection cutoff. The results show that species-specific probes perform well in the presence of nonspecific background DNA, which is expected when analyzing PDX models. The data show that the mouse component of PDX models can be analyzed using the Infinium Mouse Methylation BeadChip, while the human component can be assessed using the Infinium MethylationEPIC BeadChip.

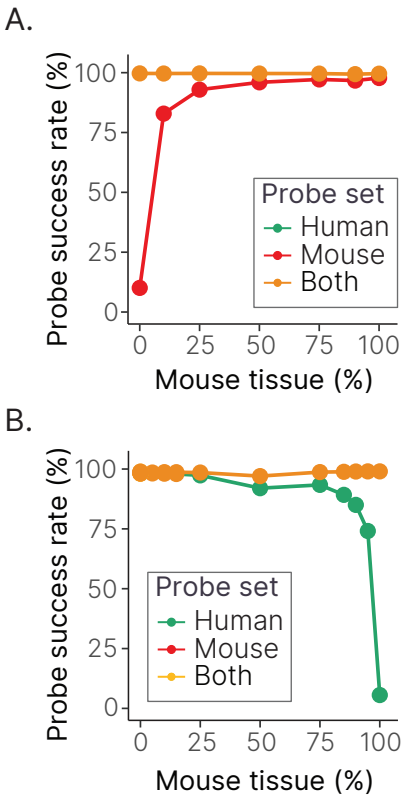

Figure 5: Probe success rates for mouse and human BeadChips—Titrated mixtures of human and mouse DNA were assayed on (A) Infinium Mouse Methylation BeadChips and (B) Infinium MethylationEPIC BeadChips. Probe performance is shown as a function of the percentage of mouse DNA included in the sample. Other than probes predicted to function for both species, no other probes pass detection when 100% human DNA is applied to the Mouse Methylation BeadChip or when 100% mouse DNA is applied to the MethylationEPIC BeadChip. When mixtures with increasing amounts of mouse or human DNA are applied to the Mouse Methylation and MethylationEPIC BeadChips, respectively, the probe success rates increase as expected.

Decitabine methylation quantification

5-aza-2'-deoxycytidine (decitabine, DAC) is an inhibitor of DNA methylation and is regularly used in experiments as a tool to modify DNA methylation levels.[11,12] To make sure that such changes can be measured with the Infinium Mouse Methylation BeadChip, clonal mouse embryo cells (ATCC cell line: C3H/10T1/2 clone 8 10T1/2; Catalog no. CCL-226) were treated with DAC or phosphate-buffered saline (PBS), and DNA methylation was assessed. DAC-treated cells showed measurable decreases in DNA methylation when compared to PBS-treated cells or untreated cells using the Infinium Mouse Methylation BeadChip (Figure 6).

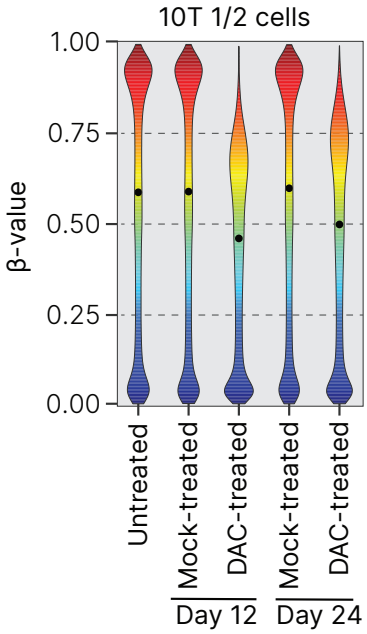

Figure 6: Methylation β-value distribution for mouse 10T1/2 cells treated with DAC or mock treated with PBS—Cells were treated for 24 hr and assayed using the Infinium Mouse Methylation BeadChip 12 and 24 days following treatment.

Conclusions

The Infinium Mouse Methylation BeadChip was designed for research applications that require broad coverage of the mouse methylome and the ability to discern small differences in DNA methylation. This application note demonstrates the ability of the Infinium Mouse Methylation BeadChip to:

- Resolve DNA differences between 0% and 100% DNA methylation
- Provide highly accurate DNA methylation measurements when using the Illumina recommended sample input ranging from 250 ng to 1000 ng of DNA
- Provide usable DNA methylation measurements for sample inputs as low as 5 ng DNA
- Compare methylation patterns for different tissue types
- Assess methylation patterns in FFPE-derived DNA samples
- Detect mouse DNA methylation changes in the presence of significant amounts of human DNA, which is a common occurrence in PDX samples
- Analyze changes in DNA methylation induced by exogenous chemicals, including DAC

The Infinium Mouse Methylation BeadChip is suitable for cancer and common disease research studies. The technical performance of the BeadChip allows for robust methylome analysis, even with low amounts of DNA input. The Infinium Mouse Methylation BeadChip fills an important gap for epigenetics assays giving researchers a powerful tool that is both cost effective and suitable for large-scale studies of disease models in mice.

Learn more

Infinium Mouse Methylation BeadChip, illumina.com/products/by-type/microarray-kits/infinium-mouse-methylation.html

References

1. Weber M, Hellmann I, Stadler MB, et al. Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat Genet*. 2007;39(4):457-466. doi:10.1038/ng1990
2. Mikeska T, Craig JM. DNA methylation biomarkers: cancer and beyond. *Genes (Basel)*. 2014;5(3):821-864. Published 2014 Sep 16. doi:10.3390/genes5030821
3. Cedar H, Bergman Y. Programming of DNA methylation patterns. *Annu Rev Biochem*. 2012;81:97-117. doi:10.1146/annurev-biochem-052610-091920
4. Klutstein M, Nejman D, Greenfield R, Cedar H. DNA Methylation in Cancer and Aging. *Cancer Res*. 2016;76(12):3446-3450. doi:10.1158/0008-5472.CAN-15-3278
5. Maros ME, Capper D, Jones DTW, et al. Machine learning workflows to estimate class probabilities for precision cancer diagnostics on DNA methylation microarray data. *Nat Protoc*. 2020;15(2):479-512. doi:10.1038/s41596-019-0251-6
6. Heiss JA, Brennan KJ, Baccarelli AA, et al. Battle of epigenetic proportions: comparing Illumina's EPIC methylation microarrays and TruSeq targeted bisulfite sequencing. *Epigenetics*. 2020;15(1-2):174-182. doi:10.1080/15592294.2019.1656159
7. Zhou W, Hinoue T, Barnes B, et al. DNA Methylation Dynamics and Dysregulation Delineated by High-Throughput Profiling in the Mouse. *bioRxiv*. 2022;03.24.485667. doi: https://doi.org/10.1101/2022.03.24.485667
8. Zhou W, Triche TJ Jr, Laird PW, Shen H. SeSAMe: reducing artifactual detection of DNA methylation by Infinium BeadChips in genomic deletions. *Nucleic Acids Res*. 2018;46(20):e123. doi:10.1093/nar/gky691
9. Triche TJ Jr, Weisenberger DJ, Van Den Berg D, Laird PW, Siegmund KD. Low-level processing of Illumina Infinium DNA Methylation BeadArrays. *Nucleic Acids Res*. 2013;41(7):e90. doi:10.1093/nar/gkt090
10. Illumina. Infinium HD Methylation Assay Reference Guide. support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/infinium_assays/infinium_hd_methylation/infinium-methylation-assay-reference-guide-15019519-07.pdf. Published Sep 2020. Accessed October 26, 2022.
11. Taylor SM, Jones PA. Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. *Cell*. 1979;17(4):771-779. doi:10.1016/0092-8674(79)90317-9
12. Yang X, Han H, De Carvalho DD, Lay FD, Jones PA, Liang G. Gene body methylation can alter gene expression and is a therapeutic target in cancer. *Cancer Cell*. 2014;26(4):577-590. doi:10.1016/j.ccr.2014.07.028

illumina®

1.800.809.4566 toll-free (US) | +1.858.202.4566 tel
techsupport@illumina.com | www.illumina.com

M-GL-00016 v1.0