High-Throughput DNA Methylation Profiling with VeraCode® Technology

Custom multiplexed DNA methylation profiling is enabled by deploying the proven GoldenGate® Assay for Methylation on high-throughput VeraCode technology.

INTRODUCTION

DNA methylation is an epigenetic modification that has significant and widespread affects on gene expression. Many studies have shown the importance of regulated patterns of methylation for normal development and function[1]. Likewise, dysregulation of methylation has been shown to have consequences leading to diseases such as cancer, MS, diabetes, and schizophrenia[2,3].

DNA methylation biomarker analysis has been hampered by the lack of an economical, mid-multiplex, high-thoughput method for profiling methylation status. Illumina's GoldenGate Assay for Methylation provides sensitive detection of methylation status at single-site resolution, and supports high multiplexing levels. With this assay deployed on VeraCode technology, researchers have an optimal combination of assay accuracy and high sample throughput. A focused set of up to 384 CpG loci can be profiled simultaneously, and hundreds of samples can be read per day with the BeadXpress® Reader (*Figure 1*).

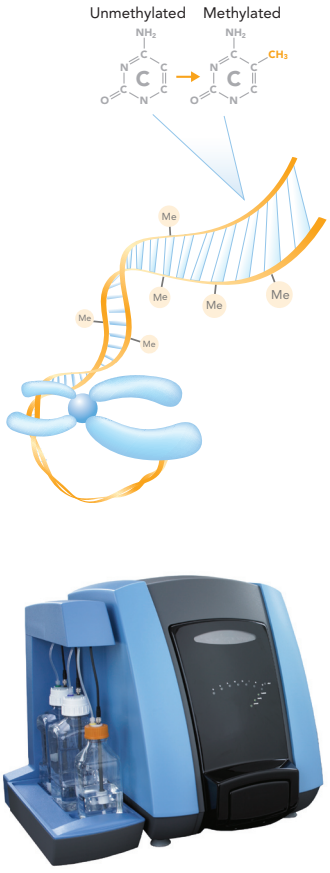
HIGHLIGHTS OF THE VERACODE METHYLATION ASSAY

- **Flexible Multiplex:** Custom assays to target 48–384 regions in a single reaction
- **Specific:** Single-site resolution
- **High Sample Throughput:** Solution-phase kinetics and streamlined assay protocol support processing hundreds of samples per day
- **Proven Accuracy:** Well-established GoldenGate Assay for Methylation is rigorously tested on the VeraCode platform
- **Convenient Workflow:** Beads provided in pre-kitted 96-well plates

VERACODE BEADS AND THE BEADXPRESS READER

The VeraCode platform leverages cutting-edge holographic bead technology to enable flexible multiplex assay deployment, analyzed at high throughput rates by the BeadXpress Reader. VeraCode microbeads are glass cylinders and each bead type is uniquely identified by an inscribed holographic code. Beads can be functionalized with bioassays for a variety of analyte types. VeraCode beads with immobilized universal capture oligonucleotides are an ideal high-throughput readout platform for the GoldenGate Assay for Methylation. Bisulfite conversion and GoldenGate Assay chemistry proceed the same way that they have for years on the BeadArray™ platform, but VeraCode solution-based kinetics support a much faster assay protocol, reducing total assay time to two days after bisulfite conversion.

FIGURE 1: DNA METHYLATION PROFILING ON THE BEADXPRESS READER



DNA methylation is an epigenetic modification where a methyl group is covalently attached to cytosine C^5 (top). The BeadXpress Reader (bottom) scans thousands of VeraCode beads per minute for their inscribed identifying codes and assay results.

The BeadXpress Reader is an automated fluidics and multi-laser imaging device that rapidly scans the bioassays deployed on VeraCode beads. Beads in solution are delivered to an internal groove plate and are scanned by the Reader for data acquisition. Individual methylation assay signals are quantified by the standard fluorescent readout of the GoldenGate Assay for Methylation while the assayed locus is identified by the code image within the bead.

GOLDENGATE ASSAY FOR METHYLATION

Illumina's GoldenGate Assay for Methylation is based on genotyping of bisulfite-converted genomic DNA (*Figure 2*). Ideal for focused studies or biomarker validation, this assay procedure is similar to that described previously for standard SNP genotyping[4], with a few modifications[5].

Using a multiple-probe design, the assay accurately differentiates between methylated and unmethylated sequences. High specificity is achieved by hybridization followed by two enzymatic discrimination steps: allele-specific extension and ligation. DNA targets are generated using a pair of allele-specific (ASO) and locus-specific (LSO) oligonucleotides. Amplification using universal primers creates products that are hybridized to specific VeraCode beads at sites bearing complementary address sequences. Amplification also incorporates a fluorescent label that denotes a methylated or unmethylated state for a given assay. Methylation status of the interrogated CpG site is then calculated as the ratio of signal from a methylated probe relative to the sum of both methylated and unmethylated probes. This value, known as β, ranges continuously from 0 (unmethylated) to 1 (fully methylated).

Custom Assay Design

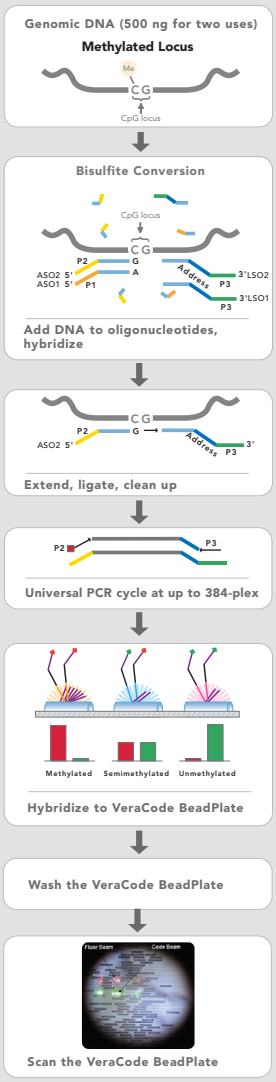
Custom Methylation Assays are easily designed to target specific CpG loci near genes or in regions of interest. Illumina scientists and a proprietary Assay Design Tool help researchers create successful content panels. Custom content designs can be submitted using any of the following formats: sequence, accession number, gene symbol, gene ID, GI number, or chromosomal region. For more information about custom content submission, please contact Illumina technical support or read the technical note, *Designing Custom GoldenGate Methylation Profiling Panels*[6].

HIGH-QUALITY DATA

The GoldenGate Assay for Methylation with VeraCode technology has been subjected to rigorous functional testing to ensure that Illumina continues to deliver the highest quality assays. These tests have shown that the methylation assay performs equally well on the VeraCode technology as on the extensively characterized BeadArray platform. Experiments comparing methylation assays across platforms show high concordance between results for the same loci on BeadArray and VeraCode substrates ($r^2 = 0.97$, *Figure 3*).

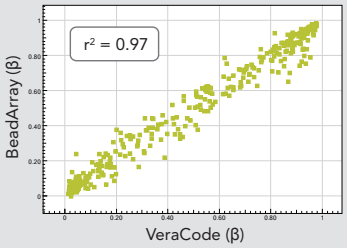
Furthermore, each bead type is present in average 30-fold redundancy to drive high accuracy and signal-to-noise ratio. This also contributes to the high assay reproducibility seen in replication experiments using GoldenGate Methylation Assays on VeraCode technology ($r^2 > 0.99$, *Figure 4*).

FIGURE 2: GOLDENGATE ASSAY FOR METHYLATION WORKFLOW WITH VERACODE TECHNOLOGY



Bisulfite-treated genomic DNA is mixed with assay oligos. Each ASO is complementary to either the converted U (unmethylated site) or the protected C (methylated site). Following hybridization, primers are extended and ligated to one of two LSOs (corresponding to a methylated or unmethylated site), creating a template for universal PCR. Labeled PCR primers are used to create a detectable product. Individual assays localize to specific VeraCode bead types by hybridization of address sequences to universal capture oligos.

FIGURE 3: ASSAY CONSISTENCY ON BEADARRAY AND VERACODE PLATFORMS



The GoldenGate Assay for Methylation gives highly consistent results across product platforms. This confirms assay quality, and provides researchers flexibility in experimental design.

Built-In Controls

To ensure assay quality in every experiment, each sample well has a set of internal controls for sample-dependent and sample-independent quality control. The BeadStudio Methylation module contains an integrated Controls Dashboard for easily monitoring graphic displays of quality control metrics.

HIGH THROUGHPUT

Unlike other high-throughput DNA methylation profiling systems, VeraCode technology supports multiplex levels up to 384 CpG loci per sample. Individual bead types can be mixed in unlimited combinations to support flexible assay design tailored to specific applications.

Faster Sample Processing

The complete GoldenGate Assay for Methylation protocol duration has been reduced and can be completed in two days after bisulfite conversion, due to solution-based kinetics and easier washing of the VeraCode beads. The streamlined workflow in 96-well format allows a single user to process hundreds of samples per day.

Pre-Kitted Bead Plates

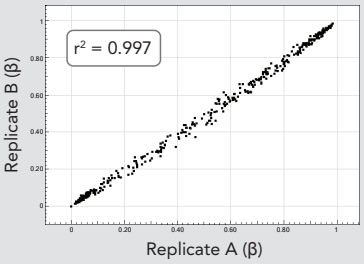
For ultimate ease and to support maximal throughput rates, Illumina provides beads pre-kitted in 96-well microtiter plates. This high-throughput workflow minimizes hands-on time and improves consistency. Users simply add the custom-designed Oligo Pool for Methylation Assay (OMA) and start the assay.

ANALYSIS SOFTWARE

Like all Illumina products, methylation assay results are collected and analyzed by the powerful Illumina BeadStudio analysis software. BeadStudio features an intuitive graphic interface for easy interpretation of results (*Figure* 5). The BeadStudio Methylation Module offers an integrated set of powerful analysis tools for characterizing, measuring, and visualizing methylation profiling results.

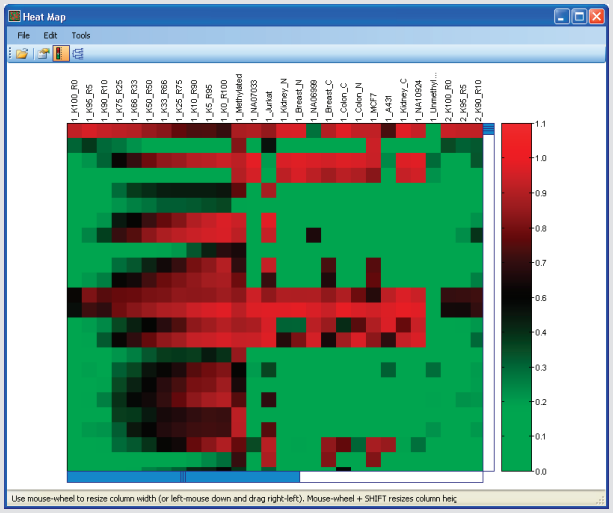
Illumina's methylation assay technology and design are consistent with other Illumina applications, including gene expression profiling. This enables researchers to perform cross-application analysis such as integrating gene expression data with DNA methylation data in BeadStudio.

FIGURE 4: HIGH ASSAY REPRODUCIBILITY



Users can be confident of robust data when using the GoldenGate Assay for Methylation with VeraCode Technology. This representative replication experiment has an r² correlation of 0.997.

FIGURE 5: DATA ANALYSIS WITH ILLUMINA BEADSTUDIO SOFTWARE



Methylation status (β) is shown for many loci (y-axis) across many samples (x-axis) in graphical heat map format to reveal trends and outliers.

BEADXPRESS PLATFORM

The BeadXpress platform provides unparalleled flexibility for designing low- to mid-multiplex, high-throughput assays. Versatile VeraCode beads support a wide variety of assay types with only minor differences between workflows. With just a single system, users can design experiments leveraging a broad portfolio of available products: the GoldenGate Genotyping Assay, protein-based assays using Carboxylated VeraCode Beads, the DASL® Assay for gene expression profiling, or the GoldenGate Assay for Methylation profiling.

ILLUMINA EPIGENETICS

Understanding the full impact of DNA methylation requires robust and flexible technologies. Illumina has developed a portfolio of products with unprecedented capabilities for studying DNA methylation—covering the spectrum from genome-wide scans to focused studies and biomarker validation—to provide unique insights into the role of epigenetics in gene regulation. For example, the HumanMethylation27 BeadChip, based on the Infinium® Assay for Methylation, offers genome-wide profiling of more than 27,000 CpG loci in 12 samples at a time. At the other end of the spectrum, creating custom methylation profiling panels that leverage the GoldenGate Assay and VeraCode technology provides researchers high-throughput focused analysis rapidly and efficiently across many more samples. Combined, these products provide researchers an end-to-end solution for biomarker discovery and validation based on DNA methylation patterns.

ORDERING INFORMATION

PRODUCT NO.	PRODUCT	BEAD TYPE MULTIPLEX*	# SAMPLES
VC-201-3096	VeraCode GoldenGate Methylation Analysis Kit	96-plex	480 samples
VC-201-3384	VeraCode GoldenGate Methylation Analysis Kit	384-plex	480 samples
Related Products			
D5004†	EZ-96 DNA Methylation Kit		2x96 reactions (deep-well format)

*Refers to number bead types. 1 or 2 beads may be used to assay each targeted region.
†Kit ordered directly from Zymo Research http://www.zymoresearch.com.

REFERENCES

(1) Ladd-Acosta C, Pevsner J, Sabunciyan S, Yolken RH, Webster MJ et al. (2007) DNA methylation signatures within the human brain. Am J Hum Genet 81: 1304-1315.
(2) Laird PW (2003) The power and the promise of DNA methylation markers. Nat Rev Cancer 3: 253-266.
(3) Egger G, Liang G, Aparicio A, Jones PA (2004) Epigenetics in human disease and prospects for epigenetic therapy. Nature 429: 457-463.
(4) Fan JB, Gunderson KL, Bibikova M, Yeakley JM, Chen J et al. (2006) Illumina universal bead arrays. Methods Enzymol 410: 57-73.
(5) Bibikova M, Lin Z, Zhou L, Chudin E, Garcia EW et al. (2006) High-throughput DNA methylation profiling using universal bead arrays. Genome Res 16: 383-393.
(6) http://www.illumina.com/downloads/CustomMethylationTechNote.pdf

ADDITIONAL INFORMATION

To learn more about the high-throughput VeraCode platform or the GoldenGate Assay or Infinium Assay for Methylation, visit our website or contact us at the address below.

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Pub. No. 170-2007-012 Current as of 16 September 2008