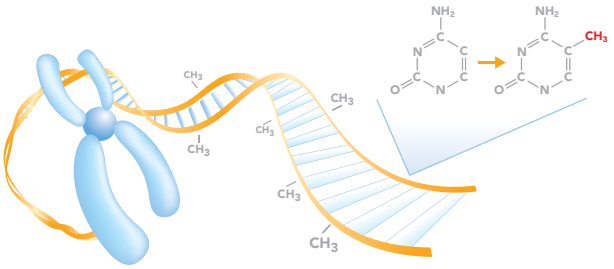
High-Throughput DNA Methylation Profiling with Illumina GoldenGate® Technology

The GoldenGate Methylation Cancer Panel I or flexible custom-designed panels support unprecedented methylation research and biomarker discovery by screening up to 1,536 independent CpG sites per assay with 96-sample throughput.

Highlights of the GoldenGate Methylation Technology

- **High Multiplex Array Platform:** up to1,536 CpG sites can be analyzed simultaneously
- **Streamlined Workflow:** 96 samples per Sentrix Array Matrix with convenient 3-day GoldenGate Assay workflow
- **Flexible Content Selection:** standard and custom panels
- **Robust Assay Performance:** high sensitivity and reproducibility (Dβ ≥ 0.2 with 95% confidence; average $r^2 \geq 0.98$)
- **Integrated Data Analysis:** comprehensive and visual data comparison
- **Versatile Platform:** SNP genotyping, gene expression, copy number variation studies, and methylation all on one platform

Figure 1: DNA Methylation Basics



DNA methylation is one of several epigenetic modifications. It involves the covalent addition of a methyl group to the 5'-carbon of cytosine in a CpG dinucleotide.

Introduction

The coupling of methylation profiling with Illumina's GoldenGate Genotyping Assay renders a powerful, high-throughput method for simultaneously analyzing the methylation status of hundreds of pre-selected genes. DNA methylation refers to the covalent addition of a methyl group to the 5'-carbon of cytosine in a CpG dinucleotide (Figure 1). Although CpG sites are located throughout the genome, a CpG Island refers to a region of at least 200 bp with increased GC content. CpG islands tend to be found in promoter regions, the first exons of housekeeping genes, and other frequently expressed genes[1].

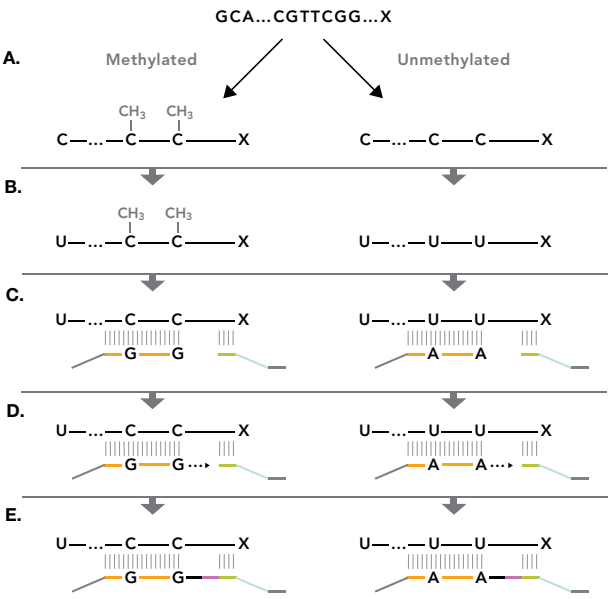
Methylation is a form of epigenetic modification that does not affect the primary structure of the genetic code, but affects secondary interactions that play a critical role in the regulation of gene expression. Aberrant DNA methylation may suppress transcription and subsequently gene expression which has been implicated in such diseases as cancer, multiple sclerosis, diabetes, and schizophrenia[1,2].

Illumina's methylation platform provides a powerful means of assessing the methylation status of up to 1,536 independent CpG sites per assay across 96 samples on either standard or custom content oligo sets. Illumina's profiling technology advances methylation research and biomarker discovery with high reproducibility (average $r^2 \geq 0.98$), flexible content design, and strong correlation to methylation-specific PCR with the sensitivity to detect small changes in methylation status between biological samples. Differential methylation analysis can now be accomplished with confidence in less than a week from bisulfite conversion to data analysis completion.

GoldenGate Assay for Methylation

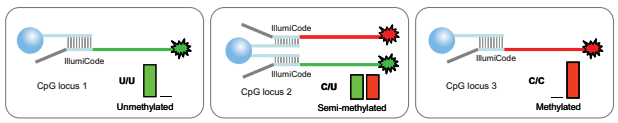
Illumina's GoldenGate Genotyping Assay has been adapted for DNA methylation detection, based on "genotyping" bisulfite-converted genomic DNA (Figure 2). Unmethylated cytosines are converted to uracils when DNA is treated with bisulfite, while methylated cytosines remain unchanged (Figure 2b). Illumina's BeadArray™ technology combines a miniaturized, bead-based array platform with a high level of assay multiplexing. The assay procedure is similar to that described previously for standard SNP genotyping[3] with a few modifications[4]. Using a four-probe design, the assay is able to differentiate between methylated and unmethylated sequences. Through allele-specific extension and ligation, DNA targets are generated using a pair of

Figure 2: GoldenGate Assay For Methylation Process Flow



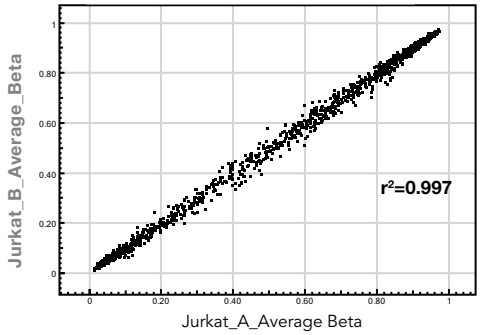
(a) Only the top strand of the gDNA sequence of interest is shown. If other CpG sites are present in close vicinity of the target CpG site, it is assumed that they have the same methylation status as the site of interest. (b) Through a bisulfite conversion step, unmethylated cytosines are converted to uracils, while the methylated cytosines remain unchanged. (c) For each CpG site, two pairs of probes are designed: an allele-specific oligo (ASO in gold) and locus-specific oligo (LSO in green) probe pair for the methylated state of the CpG site and a corresponding ASO-LSO pair for the unmethylated state. Pooled oligos anneal to the target sequence. All loci are assayed simultaneously. (d) Extension occurs from the matched ASO toward the LSO. (e) Ligation (purple) of the extended ASOs to their corresponding LSOs creates PCR templates. The ligated products are then amplified by PCR using fluorescently labeled common primers, and hybridized to a bead array bearing the complementary address sequences.

F.



allele-specific (ASO) and locus-specific (LSO) oligonucleotides (Figure 2c-2e). Amplification using universal primers occurs and the resulting products are then hybridized to a bead array at sites bearing a complementary address sequences (Figure 2f). These hybridized targets contain a fluorescent label that denotes a methylated or unmethylated state for a given locus. Methylation status of the interrogated CpG site is then calculated as the ratio of fluorescent signal from one allele relative to the sum of both methylated and unmethylated alleles. This value, also known as the β value, ranges from 0 (unmethylated) to 1 (fully methylated). The GoldenGate Assay uses several different control types to ensure the highest quality data, and each bead type is represented with an average 30-fold redundancy to further enhance the assay's accuracy.

Figure 3: Customer0Generated Reproducibility Data Across Replicates



The high reproducibility observed when using Illumina's GoldenGate Assay for Methylation is exemplified by the two Jurkat (Human Acute T-Cell Leukemia Cell Line) replicates shown.

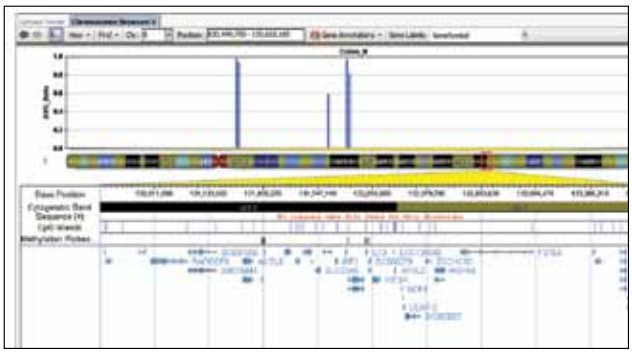
Goldengate Methylation Cancer Panel I

The first standard panel, GoldenGate Methylation Cancer Panel I, spans 1,505 CpG loci selected from 807 genes where 28.6% contain one CpG site per gene, 57.3% contain two CpG sites, and 14.1% have three or more sites. Selected genes fall into various classes, including tumor suppressor genes, oncogenes, genes involved in DNA repair, cell cycle control, differentiation, apoptosis, X-linked, and imprinted genes. All the genes on the GoldenGate Methylation Cancer Panel I are on the Illumina Human-6 v2 Expression BeadChip (BD-25-113 Human-6 v2 Expression BeadChip Kit- 2 pack).

Custom Methylation Panels

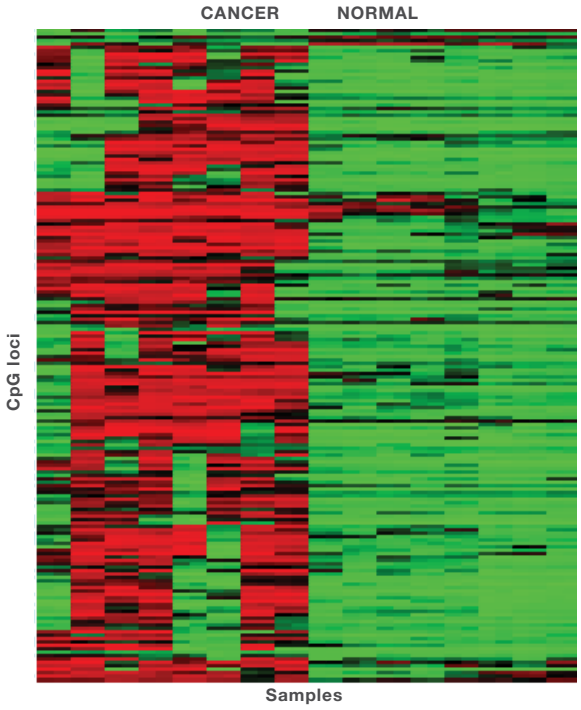
Customers can select assays to target CpG loci within genes or regions of interest. Illumina scientists will evaluate customer submissions by assigning a design score. Once all of the assays have been evaluated, a list is sent to the customer for final approval before the

Figure 4: Beadstudio's Chromosome Browser



The BeadStudio Methylation Module allows for the visualization of data along the length of the genome. The Illumina Chromosome Browser can display parameters such as beta values, methylation probes, CpG islands, and gene annotation.

Figure 5: Customer-Generated Heat Map



Using the BeadStudio heat map feature, it is easy to detect distinctly different methylation patterns at a given CpG site between normal and cancer samples.

Oligo Pool for Methylation Assay (OMA) is manufactured. Additional custom content submission can be submitted using any of the following formats: sequence, accession number, gene name, gene ID, GI number, and chromosomal region. For more information about custom content sub-mission please contact Illumina technical support.

Beadstudio Data Analysis Software

Illumina's convenient and cost-effective methylation technology is based on the versatile BeadArray platform, which powers a broad range of applications including SNP genotyping, gene expression profiling, copy number variation analysis, and methylation analysis. This enables researchers to perform cross-application analysis such as the ability to integrate gene expression data with DNA methylation data. The BeadStudio software features advanced visualization tools that enable researchers to view vast amounts of data in a single graph such as heat map, scatter plot, and line plot. These tools and the BeadStudio Genome Browser (Figure 4) show valuable information such as chromosomal coordinates, percent of GC content, location in a CpG Island, and methylation values. Figure 5 shows the utility of BeadStudio's heat map tool. This subsection of the heat map displays methylation profiles of bisulfite-converted gDNA from normal and cancerous cell lines. Methylation status of the normal samples versus samples taken from various cancer backgrounds are easily discerned by comparing methylation profiles.

Figure 6: Sentrix Array Matrix

Illumina's Sentrix Array Matrix (SAM) is capable of processing 96 samples in parallel.

SUMMARY

The Illumina GoldenGate Assay for Methylation, in conjunction with the proven BeadArray technology, creates a novel opportunity for investigators to cost-effectively survey genome-wide methylation profiles with high confidence. This is the only commercial platform that combines high sample throughput (96 samples) and high feature density (up to 1,536 CpG sites). Oligo sets can be customized and optimized with the assistance of Illumina's Assay Design Tool (ADT) to meet researchers' individual experimental goals.

Hypermethylation of CpG islands located in the promoter regions of tumor-suppressor genes is now firmly established as the most frequent mechanism for gene inactivation in cancers[4-6]. The GoldenGate Assay for Methylation, with its sophisticated data analysis tools, will provide powerful insight into epigenetic mechanisms of gene regulation. This advancement may provide much needed knowledge to further clarify genetic mechanisms underlying many of today's most common diseases.

References

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Ordering Information

Illumina catalog No.	Product	Description
GM-17-211	Methylation Cancer Panel I	Experimentally validated Oligo pool for Methylation Assay (OMA) for 1,505 CpG sites selected in promoter regions and/or first exon of over 800 genes. Sufficient for the analysis of 96 samples.
GM-95-201	Single-Use Activation Kit* (576 Samples)	Used in combination with the GoldenGate Assay Kit. Contains reagents for six 96-well plates of samples.
GM-95-205	GoldenGate Assay Kit with UDG* (96 Samples)	Prepares genotyping reactions for 96 DNA samples. Contains UDG enzyme for contamination control.
GM-12-109	Sentrix Universal 96-Array Matrix (1,536-plex) Figure 6	Processes 96 samples with up to 1,536 assays per sample.

* Multiple-use and non-UDG kits are also available. Please contact your Illumina sales representative for details.

Related products	Product	Description
Cat# D5004	EZ-96 DNA Methylation Kit‡ (2 x 96 DNA conversion reactions)	EZ DNA Methylation Kit is a bisulfite method for DNA methylation analysis of 2 x 96 samples.

‡The EZ DNA Methylation Kit must be ordered directly from Zymo Research http://www.zymoresearch.com/. Also available are Cat#D5001 for 50 DNA conversion reactions and Cat#D5002 for 200 DNA conversion reactions.

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