GENOMICS INFORMATICS PROTEOMICS METABOLOMICS

ATCTGATCCTTCTGAACGGAACTAATTTCAA
GAATCTGATCCTTGAACTACCTTCCAAGGTG

The Agilent DNA Methylation Microarray Application

Epigenome Mapping at High-Resolution

Agilent has a new microarray-based platform for studying methylated DNA that leverages our proven high-performance SurePrint inkjet synthesis technology. Array-based DNA methylation is the latest addition to our integrated and comprehensive repertoire of proven genomics tools. Create high-resolution, genome-wide methylation profiles by using robust and highly sensitive microarrays. Our platform combines our established promoter tiling microarrays with the first commercially available microarrays built with content specifically focused on CpG Islands. This comprehensive solution lets you develop a clearer picture than ever before and gain insight into mammalian DNA methylation and gene regulation.

"We have been using Agilent CpG Island arrays for custom epigenetic assays as part of our major ENCODE project effort to annotate the functional elements of the human genome. We do not have to amplify our sample since such a small amount of material is required, and the long probe lengths allow for greater specificity and excellent replicability."

– David Johnson, PhD
Director,
Myers-Stanford ENCODE Project

Introduction

Epigenetics is the study of heritable modifications that regulate gene expression but do not change DNA sequence itself. In humans, DNA methylation is a primary epigenetic modification known to be closely involved in widespread and fundamental processes such as cancer, development, genomic imprinting, gene silencing, and chromatin stability.

In vertebrates, DNA methylation patterns are clustered in "CpG Islands," CG-rich regions with stretches where the frequency of the "CG" sequence is higher than in surrounding regions. In somatic cells, CpG Islands are usually unmethylated, positioned at the 5' ends of many genes, and often in promoter regions (Bird 1987). About 60% of all gene promoters have CpG Islands (Antequera and Bird 1993). Recent computational analysis identifies ~28,500 CpG Islands in the human genome and 16,020 in that of the mouse (UCSC Genome Browser). During development, some Islands are methylated, enabling the recruitment of repressive complexes (such as polycomb) that silence the associated promoters.

Gene expression pattern changes resulting from altered or aberrant DNA methylation within CpG Islands have been correlated with various cancers and as a hallmark of disease progression. Understanding global methylation is invaluable and holds great promise in driving the understanding (and identification of epigenetic markers) in disease pathology and progression, as well as vertebrate development.

New Powerful Tools

Conventional methods of analyzing DNA methylation (such as bisulfite modification-based mapping and methylation-specific PCR) are low-throughput, labor intensive, and expensive. Microarray-based methods have recently evolved as powerful high-throughput analysis tools capable of detecting and mapping DNA methylation changes on a previously unachievable genome-wide scale. Agilent is uniquely able to offer comprehensive arrays—specifically focused on CpG Islands—that deliver relevant and up-to-date content.

Features and Benefits

Superior Microarray Performance

Agilent's microarrays deliver the optimal sensitivity and specificity. Proprietary microarray technology using 60-mer oligonucleotide probes and a convenient two-color labeling system delivers higher sensitivity, accuracy, and greater reproducibility than other competitive platforms. These unique features allow sensitive measurements as well as direct comparisons of samples on the same microarray.

The Agilent Probe Advantage

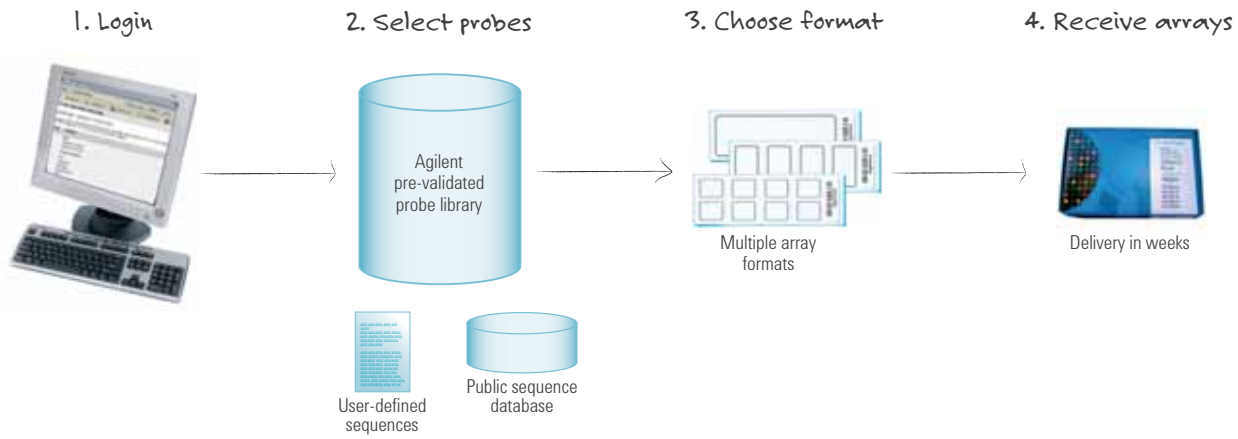
Our Promoter 2-set microarrays have the high signal-to-noise ratios essential for the success of DNA methylation experiments due to optimized and validated probe design. Unlike other companies, Agilent carefully designs probes using stringent criteria and does not sacrifice clean, robust data for higher-density microarrays.

For Agilent CpG Island microarrays, probes were selected for uniqueness within the genome and predicted hybridization properties according to standard Agilent probe design criteria (with the exception of the theoretical T_m window restriction). For some probes, due to the high CG content in CpG Islands, the standard T_m restriction was lifted to achieve appropriate spacing.

- 60-mer oligonucleotide probes provide robust hybridization–critical for the sensitivity and specificity that DNA methylation demands.
- Average probe spacing parameters are specifically optimized for the DNA methylation application as compared to other microarray methods.
- Repeat regions are masked to significantly reduce nonspecific noise.

* Probe design criteria includes optimal T_m, unique sequence, and self-structure prediction.

Customize Your Expression Tools and Optimize Your Results



Freedom to Build Your Own Arrays

eArray, the free web-based design tool, gives you the power to create your own custom microarray, quickly and easily. Build an array based on your own imported sequences and with our no-cost probe design, or use our extensive database of validated and optimized probes and annotations. Agilent's SurePrint inkjet technology ensures rapid design manufacture so you can receive your custom microarrays in weeks, anywhere in the world.

Agilent SurePrint Technology

SurePrint features a flexible, industrial scale inkjet printing process that synthesizes 60-mer oligonucleotide probes directly onto the array, resulting in high-purity, high-fidelity probes. The maskless process allows quick iteration of microarray designs required in today's rapidly evolving epigenomics field. This provides you with easy access to high-quality arrays loaded with useful, rich content.

Scale Experiments to Meet Your Research Needs

Agilent's SurePrint technology and printing formats are key elements of the Agilent integrated platform. Custom tailor your own design for any and all of your DNA methylation studies by choosing probes from our optimized human, mouse, and rat databases tiled at high resolution across each genome. Depending on your desired level of focus, you can design 1, 2, 4, or 8 microarrays per slide.

Compatible With Various Methods

The Agilent CpG Island microarray has been designed for high-resolution DNA methylation analysis using a variety of assay preparation methods. Our design is compatible with both affinity-based (Weber et al. 2005; Keshet et al. 2006; Rauch et al. 2007) as well as restriction enzyme-based (Yan et al. 2000) methods for enriching methylated DNA. A representative workflow is shown below in **Figure 1**. (Note that restriction enzyme-based methods are limited by the location of restriction sites relative to the probe locations employed in any specific strategy.)

An Integrated Platform

As the latest addition to our integrated and comprehensive portfolio of proven microarray-based genomics tools, DNA methylation profiling is synergistic with our gene expression and ChIP-on-chip microarray products. Agilent's core microarray technology encompasses sample preparation and labeling, an integrated experimental workflow, and comparison across multiple applications. By enabling you to answer complex questions at the intersection of transcriptomics and genetics, Agilent microarrays give you a more complete picture.

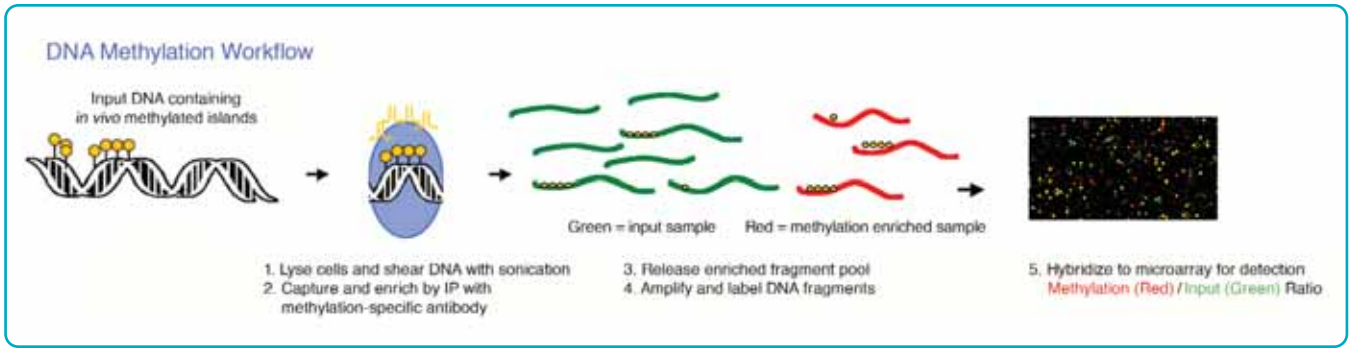


Figure 1. Affinity-based isolation of methylated DNA. A representative example of one method for isolation and enrichment of methylated DNA. Methylated regions of the genome (mDIP) from a genomic DNA sample are isolated with a monoclonal antibody to 5-methylcytosine. Isolated DNA is purified, Cyanine 5-labeled, and competitively hybridized against similarly Cyanine 3-labeled "input" genomic DNA onto a single microarray. Arrays are washed, scanned, and analyzed with Feature Extraction software. Relative DNA methylation levels for each probe/CpG Island are reflected in changes in Cyanine 5/Cyanine 3 ratios.

High-Performance Profiling

Several methods describing the enrichment of methylated DNA have been reported. Agilent's Human CpG Island microarray was used to profile and compare methylated DNA patterns, specifically at the HOXA gene cluster on Chromosome 7, in normal versus carcinoma lung cell line samples (**Figure 2**). Profiles clearly show significant differences between the normal and cancer samples at the HOXA6, HOXA7, and HOXA9 promoter regions. Similar Hox methylation patterns and putative epigenetic microdeletion genomic lesions have recently also been described in stage 1 lung carcinoma samples and breast cancer (Rauch et al. 2007; Novak et al. 2006). These discoveries are promising biomarkers for disease profiling and staging.

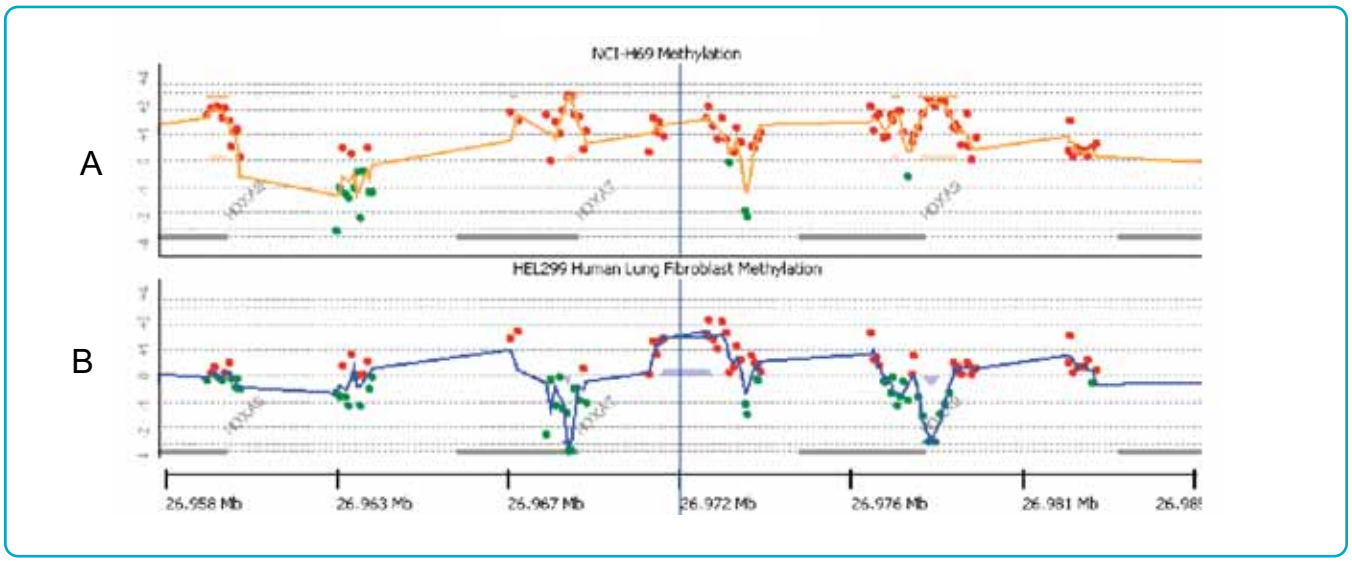
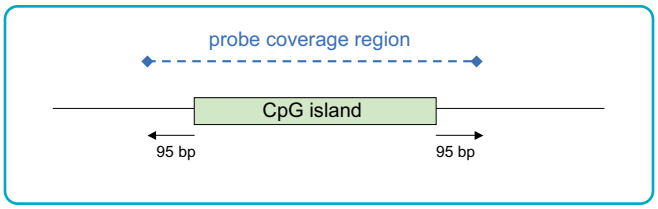


Figure 2. Comparison of lung carcinoma cell line and normal lung cell line at the HoxA gene cluster on Chromosome 7. Methylated DNA was immunoprecipitated and labeled (as described in Figure 1) from NCI-H69 small cell lung carcinoma (**A**) and HEL299 normal lung fibroblast (**B**) genomic DNA samples. Cy5-labeled methylated DNA and Cy3-labeled input genomic DNA were competitively hybridized to an Agilent Human CpG Island Microarray (P/N G4492AA). Microarrays were processed, and then scanned on an Agilent Scanner (P/N G2565BA) and data normalized to a median $\log_2$ ratio of zero. Every point represents an individual CpG Island microarray probe. Each colored line (blue for normal, orange for lung carcinoma) represents a three-point moving average of the $\log_2$ ratio. Regions of more methylation (red) and regions of less methylation (green) are depicted. Highly methylated regions have log ratios significantly above zero while less methylated regions have log ratios significantly below zero.

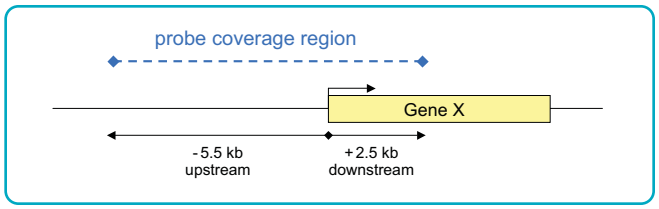
Specifications

Product	CpG Island		Promoter 2-Set	
Species	**Human**	**Mouse**	**Human**	**Mouse**
Format	1 x 244K	2 x 105K	1 x 244K	1 x 244K
Slides per kit	5	5	10	10
Samples processed per kit	5	5	5	5
Slide format	1" x 3"		1" x 3"	
Probe length	45-60mer		60mer	
Feature size	65 µm		65 µm	
Interval coverage	27,801 bp (covering 21 MB)	16,030 bp	-5.5 kb to +2.5 kb of 17,000 Ref Seq genes	
Number of data probes	~237,006	97,652	243,504	
Internal quality control probes	1,600		1,600	
Average probe spacing	~100 bp		~200 bp	
Sequence source	For human: UCSC hg18/NCBI release 36.1 (March 2006 build) For mouse: UCSC mm8/NCBI release 36 (February 2006 build)			
Starting sample	4 µg genomic DNA			
Labeling type	Random priming using Klenow with Cy3 and Cy5 nucleotides			
DNA required (hybridization)	5 µg per channel			
Hybridization volume	500 µL			

Probe coverage characteristics



Agilent CpG Island



Agilent Promoter 2-Set

Notes:

Notes:

Ordering Information	
Human CpG Island Microarray	G4492A
Mouse CpG Island Microarray	Please inquire
Human 2-Set Promoter Microarray	G4489A
Mouse 2-Set Promoter Microarray	G4490A
Hybridization Chamber	G2534A
Hybridization Gasket Slide (1 X 244K, 2 X 105K)	G2534-60003, G2534-60002

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About Agilent's Integrated Biology Solutions

Agilent Technologies is a leading supplier of life science research systems that enable scientists to understand complex biological processes, determine disease mechanisms, and speed drug discovery. Engineered for sensitivity, reproducibility, and workflow productivity, Agilent's integrated biology solutions include instrumentation, microfluidics, software, microarrays, consumables, and services for genomics, proteomics, and metabolomics applications.

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