Brief Introduction of DNA Methylation: Basic Concept and Research Methods
(adapted from: "DNA methylation –methods and protocols". 2009. ed. By J. Tost , Humana Press)

1. DNA Methylation: An Introduction to the Biology and the Disease-Associated Changes of a Promising Biomarker.

DNA methylation occurring on the 5' position of the pyrimidine ring of cytosines in the context of the dinucleotide sequence CpG forms one of the multiple layers of epigenetic mechanisms controlling and modulating gene expression through chromatin structure. It closely interacts with histone modifications and chromatin-remodeling complexes to form the genomic chromatin landscape. DNA methylation is essential for proper mammalian development, crucial for imprinting, and plays a role in maintaining genomic stability as well as in dosage compensation. DNA methylation patterns are susceptible to change in response to environmental stimuli such as diet or toxins whereby the epigenome seems to be most vulnerable during early *in utero* development. Aberrant DNA methylation changes have been detected in several diseases, particularly cancer where genome-wide hypomethylation coincides with gene-specific hypermethylation. DNA methylation patterns can be used to detect cancer at very early stages, to classify tumors as well as predict and monitor the response to antineoplastic treatment. As a stable nucleic acidbased modification with limited dynamic range that is technically easy to handle, DNA methylation is a promising biomarker for many applications.

2. Quantification of Global DNA Methylation by Capillary Electrophoresis and Mass Spectrometry

Two approaches for the evaluation of the relative degree of global DNA methylation through the quantification of 2'-deoxynucleosides are described. Detection and quantification of 5-methyl 2'-deoxycytidine in genomic DNA is performed using both high-performance capillary electrophoresis (HPCE) with UV–Vis detection or liquid chromatography with electrospray ionization mass spectrometric detection (LC-ESI/MS). Treatment of genomic DNA with a ribonuclease and generation of nucleosides through enzymatic hydrolysis notably increases the specificity of both techniques. Both approaches have been demonstrated to be highly specific and sensitive, being useful for the rapid quantification of the degree of global DNA methylation and its exploitation for the analysis of poorly purified and/or concentrated DNA samples, such as tumor biopsies.

3. Methyl Group Acceptance Assay for the Determination of Global DNA Methylation Levels

DNA methylation levels are affected by numerous environmental influences, including diet and xenobiotic exposure, and neoplasia has been firmly associated with genomic hypomethylation and localized hypermethylation of tumor suppressor genes. To reverse methylation-induced gene repression, DNA hypomethylating agents are currently in clinical trials for various malignancies, with two of these now

approved for the therapy of myelodysplastic syndrome, and the efficacy of these drugs can be assessed by the monitoring of global DNA methylation levels. Herein, we outline a simple, well-established method for the evaluation of genomic DNA methylation levels, based on the ability of isolated DNA to "accept" radiolabeled methyl groups from *S*-[^{3}H-methyl] adenosylmethionine, using the bacterial CpG methyltransferase SssI. As this enzyme methylates all unmethylated CpG dinucleotides in the genome, radiolabeled methyl group acceptance is inversely proportional to the level of preexisting methylation. This assay is applicable to a number of translational and basic research questions.

4. Immunodetection Array

A novel procedure for DNA methylation analysis is described that characterizes the extent of DNA methylation in CpG islands. The basic concept relies on direct immunodetection of 5'methylcytosines (5'mCs) without the need for bisulfite treatment utilizing a microarray format. This system is designed for the application of immunofluorescence using a monoclonal antibody that specifically recognizes 5'mC in single-stranded DNA hybridized to oligonucleotide microarrays. An ultrasensitive fluorescence scanner and 170-μm thin aldehyde-functionalized glass slides are used to optimize the signal-to-noise ratio and to minimize autofluorescence. These methodological improvements allow for the direct detection of 5'mC in genomic DNA hybridized to microarrays without prior PCR amplification with high analytical sensitivity.

5. Methylated DNA Immunoprecipitation (MeDIP)

Methylated DNA immunoprecipitation (MeDIP) is a versatile immunocapturing approach for unbiased detection of methylated DNA. In brief, genomic DNA is randomly sheared by sonication and immunoprecipitated with a monoclonal antibody that specifically recognizes 5-methylcytidine. The resulting enrichment of methylated DNA in the immunoprecipitated fraction can be determined by PCR to assess the methylation state of individual regions. Alternatively, MeDIP can be combined with large-scale analysis using microarrays as a genome-wide experimental readout. This protocol has been applied to generate comprehensive DNA methylation profiles on a genome-wide scale in mammals and plants, and further to identify abnormally methylated genes in cancer cells.

6. The MIRA Method for DNA Methylation Analysis

DNA methylation patterns are often altered in human cancer and aberrant methylation is considered a hallmark of malignant transformation. Several methods have been developed for the characterization of gene-specific and genome-wide DNA methylation patterns. In this chapter, we describe the methylated-CpG island recovery assay (MIRA), which is based on the high affinity of the MBD2b/MBD3L1 complex for double-stranded CpG-methylated DNA. MIRA has been used in combination with microarray platforms to map DNA methylation patterns across the human genome.

7. The HELP Assay

Genomic representations using ligation-mediated PCR have been used successfully as the foundation for a number of high-throughput assays. *Hpa*II tiny fragment enrichment by ligation-mediated PCR (HELP) is an example of the use of such representations to study cytosine methylation in the genome. The HELP assay differs from most other assays testing cytosine methylation because of its positive representation of hypomethylated DNA in the genome, whereas other assays infer the presence of hypomethylated sequences by the absence of signal, for which there can be confounding technical reasons. Hypomethylated sequences represent the minority of the genome and tend to be located at unique sequences with functionally interesting properties such as transcription start sites. By performing a comparative genomic hybridization using an *Msp*I representation from the same DNA sample, we represent all potential loci that could be generated by *Hpa*II in the situation of global hypomethylation; in practice, *Hpa*II generates a subset of loci from this population, allowing us to discriminate hypomethylated loci (represented by both *Hpa*II and *Msp*I) from methylated loci (represented by *Msp*I only).

8. Differential Methylation Hybridization: Profiling DNA Methylation with a High-Density CpG Island Microarray

Differential methylation hybridization (DMH) is a high-throughput DNA methylation screening tool that utilizes methylation-sensitive restriction enzymes to profile methylated fragments by hybridizing them to a CpG island microarray. This array contains probes spanning all the 27,800 islands annotated in the UCSC Genome Browser. Herein we describe a revised DMH protocol with clearly identified quality control points. In this manner, samples that are unlikely to provide good readouts for differential methylation profiles between the test and the control samples will be identified and repeated with appropriate modifications. In addition to the step-by-step laboratory DMH protocol, we also provide a detailed description regarding DMH data analysis. The suggested microarray platform contains 244,000 probes and it can be a daunting barrier for researchers with no prior experience in analyzing DNA methylation data. We have created a data analysis pipeline available in a user friendly, publicly available interface, the Broad Institute's GenePattern software, which can be accessed at http://bisr.osumc.edu:8080/gp. This permits scientists to use our existing data analysis modules on their own data. As we continue to update our analysis algorithm and approaches to integrate high-throughput methylation data with other large-scale data types, we will make these new computation protocols available through the GenePattern platform.

9. Analysis of DNA Methylation by Amplification of Intermethylated Sites (AIMS)

DNA methylation is an epigenetic modification that plays a crucial role in the control of gene expression and chromosome structure in plants and mammalian cells. Multiple types of DNA fingerprinting techniques have been developed and applied to investigate DNA methylation profiles in different experimental

settings. One of these techniques, the amplification of intermethylated sites (AIMS) is a simple approach appropriate for genome-wide estimates of DNA methylation and the discovery of specific methylated sequences. AIMS is based on the differential enzymatic digestion of genomic DNA with methylationsensitive and methylation-insensitive isoschizomers followed by restrained PCR amplification of methylated sequences. This method is appropriate to compare large series of samples and the simultaneous identification of hypo- and hypermethylation events. Applications of AIMS include the study of DNA methylation changes in cancer and aging, and the discovery of DNA methylation in a social insect.

10. Methylation-Sensitive Representational Difference Analysis(MS-RDA)

Methylation-sensitive representational difference analysis (MS-RDA) is a genome subtraction method that isolates DNA fragments differentially methylated between two genomes. It can be performed in any organism, even in those for which no microarray products are available. An important characteristic of MS-RDA is that it enriches unmethylated CpG-rich regions of the genome (amplicon), most of which are unique sequences. DNA fragments differentially methylated between two DNA samples will be present in one amplicon, but not in the other. The difference can be identified by RDA. Most technical difficulties reside in the RDA procedure, and many fine techniques are necessary for a successful application of this powerful technology.