
Preface

Epigenetics can be defined as the study of heritable changes in gene expression without alteration of the DNA sequence itself. This means that epigenetic variants are stable alterations that are heritable during somatic cell divisions (and possibly transmitted through germ line transmissions in some occasions) but do not involve mutations of the DNA itself. Epigenetic phenomena are mediated by various molecular mechanisms, including histone modifications and core histone variants; ATP-dependent chromatin-remodeling complexes; polycomb/trithorax protein complexes; small RNAs, including siRNA and miRNAs as well as other noncoding RNAs; and last but not least DNA methylation. This volume in the *Methods in Molecular Biology*TM series focuses entirely on protocols for the analysis of DNA methylation, which is the only genetically programmed DNA modification in mammals occurring almost exclusively at the carbon 5 position of cytosines followed by a guanine.

Realizing the importance of epigenetic changes in development and disease, a variety of techniques for the study of DNA methylation have been developed over the last few years. **Figure 1** gives an overview of many of the commonly used technologies, but many more methods and variants of the named assays do exist. No single method has emerged as the “gold” standard technique unifying quantitative accuracy and high sensitivity or possibilities for whole genome analysis and precise investigations of individual CpG positions. The choice of the method mainly depends on the desired application. Although by no means complete, this second edition of “DNA methylation” gives a comprehensive overview of available technologies together with detailed step-by-step protocols for all experimental procedures required to successfully perform DNA methylation analysis.

This is the second edition of the DNA methylation protocols; however, the field has dramatically changed within the 6 years that have passed since the first edition edited by K.I. Mills and B.H. Ramsahoye was published. As DNA methylation technologies and our knowledge of DNA methylation patterns have been advancing at a breathtaking pace over the past few years and most of the techniques described in the first edition have been further optimized and/or replaced by novel, easier, refined, and/or more quantitative technologies, I have entirely remodeled the contents of this book. The increase in available methods is also reflected in the great expansion of the number of chapters within this book. While the first edition contained 14 chapters, this second edition consists now of 27 chapters. Only three chapters have been retained from the first edition and these have been completely rewritten by the authors to accommodate the changes and improvements made in the last years. The analysis of gene-specific DNA methylation patterns has been complemented or superseded by genome-wide approaches and epigenomics has taken a central place in many laboratories.

The selection of different technologies enables the analysis of the global DNA methylation content as well as precise quantitative data on single CpG positions. Methods for the high-resolution analysis of CpG positions within a target region identified by one of the multiple available genome-wide technologies are presented, and emphasis has been placed on array-based approaches that permit a hypothesis-free-driven research to identify

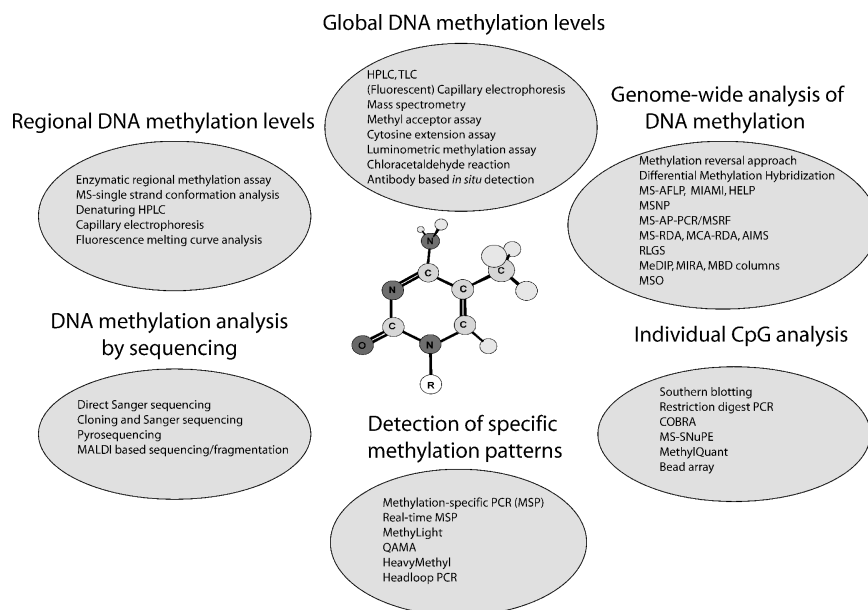


Fig. 1. An overview of the different technologies used for the analysis of DNA methylation. MS: Methylation sensitive; HPLC: High-performance Liquid Chromatography; TLC: Thin-layer Chromatography; MS-AFLP: Methylation-sensitive Amplified Fragment Length Polymorphism; MIAMI: Microarray-based Integrated Analysis of Methylation by Isochizomers; HELP: *HpaII* tiny fragment Enrichment by Ligation-mediated PCR; MSNP: Methylation Single Nucleotide Polymorphism; MS-AP-PCR: Methylation-sensitive Arbitrarily-primed PCR; MSRF: Methylation-sensitive Restriction Fingerprinting; MS-RDA: Methylation-sensitive Representational Difference Analysis; MCA-RDA: Methylated CpG island Amplification—Representational Difference Analysis; AIMS: Amplification of intermethylated Sites; RLGS: Restriction Landmark Genomic Scanning; MeDIP: Methylated DNA ImmunoPrecipitation; MIRA: Methylated CpG Island Recovery Assay; MSO: Methylation-specific Oligonucleotide array; MALDI: Matrix-assisted Laser Desorption/Ionization mass spectrometry; COBRA: Combined Bisulfite Restriction Analysis; MS-SNuPE: Methylation-sensitive Single Nucleotide Primer extension; QAMA: Quantitative Analysis of Methylated Alleles. Reproduced with permission from Tost, J. (2008) *Methods for the genome-wide and gene-specific analysis of DNA methylation levels and patterns*. In: *Epigenetics* (Tost, J., ed.), Horizon Scientific Press, Norwich, UK, pp 63–103.

DNA methylation patterns of interest. In the final chapters of this book, more specialized applications like the sensitive detection of aberrant methylation patterns in body fluids, prevention of contamination, and whole genome amplification of bisulfite-treated DNA are described. Methods requiring special instruments are presented along technologies that can be performed with a simple thermocycler. This volume of the *Methods in Molecular Biology*TM series contains widely used methods, such as cloning and sequencing and methylation-specific PCR as well as novel and promising techniques such as the immunodetection array that have only very recently passed the proof-of-principle stage.

This book is addressed to postdoctoral investigators and research scientists that are implicated in the different aspects of genetics and cellular and molecular biology as well as to clinicians involved in diagnostics or choice of treatment of diseases that have an epigenetic component. The presentation in this volume is equally suited for laboratories that already have a great deal of expertise in a certain technology to analyze DNA methylation, but might want to obtain other or complementary data using a second technique, and for genetics/genomics/biology groups that want to initiate research in this exciting area and want to identify the method best suited to answer their question. Notes and tips from

the experts and/or pioneers of the different methods will enable a rapid implementation of the different protocols in the laboratory and avoid time-consuming and cost-intensive mistakes. With the tools and protocols available, our knowledge and understanding of DNA methylation will increase rapidly, and this book will contribute to spreading of the “savoir faire” to analyze DNA methylation.

I am indebted to all the authors for their hard work and outstanding contributions to this second edition of “DNA methylation”. It was a pleasure to work with them on this project. I hope that the protocols described in detail in this volume will help to accelerate the analysis and description of the “methylome” of different species and will enhance our understanding of the molecular processes that determine the genomic DNA methylation landscape.

Evry, March 2008

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