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Review Article

Comprehensive analysis of DNA-methylation in mammalian tissues using MeDIP-chip

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ABSTRACT

Genome-wide mapping of epigenetic changes is essential for understanding the mechanisms involved in gene regulation during cell differentiation and embryonic development. DNA-methylation is one of these key epigenetic marks that is directly linked to gene expression is. Methylated DNA immunoprecipitation (MeDIP) is a recently devised method used to determine the distribution of DNA-methylation within functional regions (e.g., promoters) or in the entire genome robustly and cost-efficiently. This approach is based on the enrichment of methylated DNA with an antibody that specifically binds to 5-methyl-cytosine and can be combined with PCR, microarrays or high-throughput sequencing. This article outlines the experimental procedure of MeDIP-chip and provides a comprehensive summary of quality control strategies and primary data analysis.

1. Introduction

DNA-methylation at CpG-dinucleotides is one of the most extensively studied epigenetic marks. It modulates several genomic functions including regulation of differential gene expression, chromatin structure organization, X-inactivation, stable silencing of transposable elements, cellular differentiation, embryonic development and genomic imprinting [1,2]. Due to its molecular stability and heritability DNA-methylation is considered a key signature of epigenetic changes that can be monitored at a genome-wide level [3]. While most DNA-methylome studies have focused on detecting epigenetic modifications in a disease context such as cancer [4], there is growing interest to analyse the dynamic methylation changes that take place during cell differentiation and embryonic development on a genome-wide scale. Recent studies in mouse stem cells indicate that DNA-methylation plays a crucial role in cell differentiation from stem cells to terminally differentiated cells like neurons [5,6].

In the last years a range of methods has been developed to profile DNA-methylation on a genome-wide level [3,7–9]. Up to this date, the most comprehensive method to obtain DNA-methylation profiles at base-pair-resolution is whole genome bisulfite-sequencing [10]. This method, however, is still rather costly, as it requires a fair amount of sequencing power to generate quantitative maps. Alternative approaches are based either on immunoprecipitation, differential affinity binding or restriction enzyme selection. All these methods can be combined with microarray hybridisation to monitor DNA-methylation changes on selected genomic regions across several tissues or cell lines. The methods available are certainly comparable, but achieve different levels of coverage, sensitivity and accuracy [2,9,11]. The most widely used array-based methods reported are: (i) methylation-sensitive or methylation-specific restriction (e.g., DMH, [12]; CHARM, [11]; HELP, [13]), (ii) capturing of methylated DNA by means of methyl-binding proteins (e.g., mCIP, [14,15]; MBD-affinity purification, [16]) or (iii) immunoprecipitation of methylated DNA (e.g., MeDIP, [17,18]).

Since the establishment of MeDIP [17], the technique has emerged as one of the most widely used methods in the scientific community. Currently, commercial kits are available [19] and automated robotic systems have been implemented for large sample approaches. The method has been used to derive methylation maps in different organisms [16,20–22], for epigenomic mapping in cancer [17,18] and to monitor epigenomic changes upon differentiation of embryonic stem cells [6,23]. With recent improvements in microarray design, it is now possible to analyse up to 4% of a mammalian genome on a single microarray at a relatively low-cost. Alternatively, the methylated precipitate can be subjected to high-throughput-sequencing [24], providing a direct sequencing readout of 5mC-enriched fragments. On the other hand, the identification of regions with differential methylation

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requires an adequate genomic sequencing coverage, which is rather costly at present.

In both MeDIP applications, irrespective of whether they are based on microarrays or sequencing readout, only the relative enrichment of methylated DNA can be determined in comparison to control *Input* DNA. To obtain reproducible data and thus allow comparative interpretations of MeDIP-results, it is important to optimize and standardize both the experimental procedure and the bioinformatic processing of data. In addition, technical variation between experiments should be monitored at every step and controls must be introduced. In this article, we will describe the protocol for MeDIP with subsequent hybridization to high-density tiling arrays, used in our laboratory. In addition, we will discuss the critical aspects of the method stated above and present a general workflow for the analysis of MeDIP microarray data, including the implementation of basic bioinformatic tools for primary data evaluation.

2. Methylated DNA immunoprecipitation (MeDIP)

The MeDIP procedure is based on the enrichment of methylated DNA with an antibody that specifically binds to 5mC. It involves several steps (Fig. 1). First, high molecular genomic DNA is randomly fragmented by sonication, denatured and incubated with the anti-5mC antibody. Afterwards, purified bound methylated DNA (IP) can be evaluated for enrichment by comparing the methylated IP fraction to control sonicated DNA (Input DNA). Single loci can be analyzed by standard methods such as quantitative real time PCR (qPCR). Furthermore, on a genome-wide scale, the methylated IP fraction and Input DNA are amplified and differentially labeled to be co-hybridized on the same microarray for fold-enrichment comparison. Critical quality assessment at each of these steps is crucial to obtain relevant results. This section describes the protocol in detail and discusses major aspects that should be considered for a successful experiment. Our protocol is a modified version of the method described by Weber and collaborators [17].

2.1. Extraction of genomic DNA

To extract genomic DNA we use standard phenol–chloroform extraction methods. Briefly, tissue or cell culture samples are resuspended in 500 µl of buffer (25 mM EDTA; 75 mM NaCl) and incubated 10 min with 5 µl of RNAse (20 mg/ml). Afterwards, 500 µl of lysis buffer (10 mM EDTA; 10 mM Tris–HCl, pH 8.0; 1% SDS) are added and Proteinase K (200 µg/ml) treatment is performed overnight shaking at 55 °C. Samples are extracted twice with 1 ml of a phenol–chloroform–isoamyl alcohol mixture (25:24:1) and two more times with 1 ml of chloroform–isoamyl alcohol mixture (24:1). Aqueous phase (1 ml) is brought to 0.3 M sodium acetate and precipitated with 2.5 ml of ice-cold ethanol (100%). DNA fibers are fetched with a pipette tip and soaked briefly in ethanol (70%) to be later air-dried in a 1.5 ml microfuge tube. The DNA pellet is resuspended in 50–200 µl of 1 × TE and stored at 4 °C. Alternatively, commercial kits based on binding columns (e.g., GenElute™ Mammalian Genomic DNA Miniprep Kit from Sigma–Aldrich) can be used.

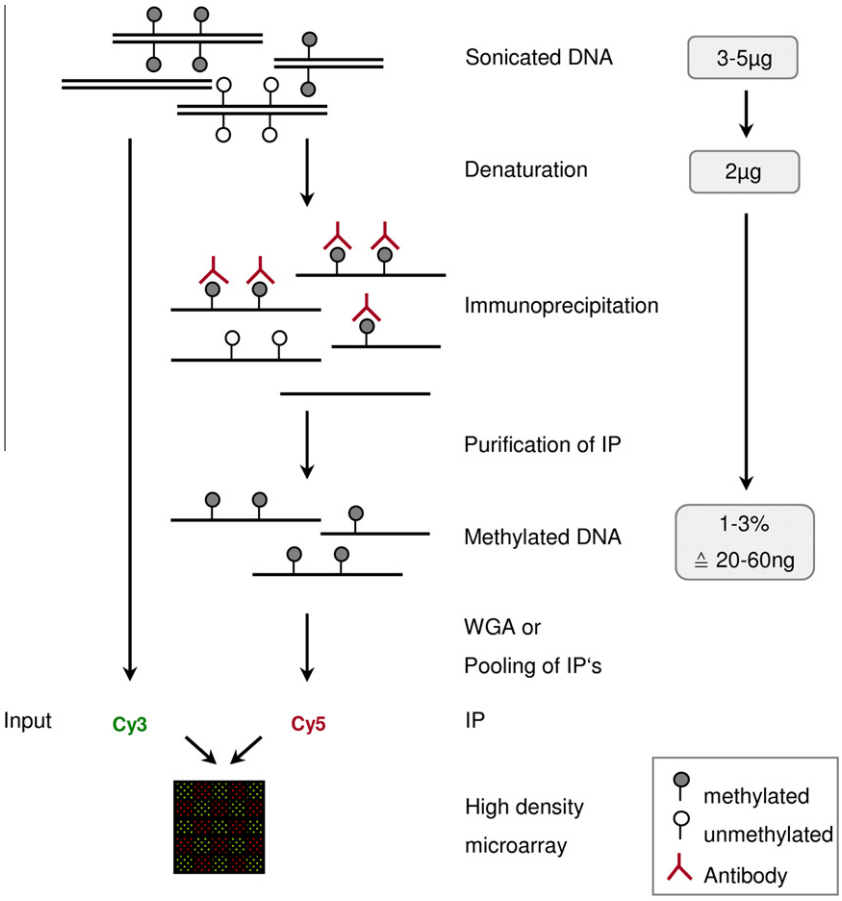

Fig. 1. Principle of MeDIP-chip. Genomic DNA is fragmented by sonication and denatured. Methylated DNA is precipitated using an antibody against 5mC. Purified methylated DNA (IP) and sonicated control DNA (Input) are whole genome amplified and labeled with Cy5 and Cy3 respectively, to be later co-hybridized on a high density microarray.