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Bisulfite modification of immobilized DNAs for methylation detection

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Abstract

We have developed a novel method for detecting DNA methylation status of multiple samples, in which the DNA samples were firstly immobilized on the slide and treated with bisulfite directly on the chip. In this experiment, DNAs of *pUC19* plasmid were restricted by the enzymes, and ligated with a linker bearing 5′-terminal acrylamide group at the sticky ends. Using universal acrylamide gel polymerization technique, a large amount of DNAs could be immobilized on the slide. The immobilized DNAs were converted by soaking the chip in bisulfite reaction mixtures for 16 h. The probes for detection of the methylation patterns of CpG sites hybridized with the converted DNAs on the microarray, and non-specifically bound probes were cleaned by electrophoresis. We have optimized the experimental conditions of both bisulfite treatment and electrophoresis to increase sensitivity and specificity. The results were further validated by bisulfite DNA sequencing. The experiments show that the method can simplify the experimental processes and increase the efficiency of the bisulfite treatment. This novel method could be used as a convenient tool to detect the methylation status of the multiple genes for a large amount of samples in the future.

Keywords: DNA microarray; Methylation; Acrylamide gel

1. Introduction

DNA methylation is introduced enzymatically by DNA methyltransferases after DNA replication, which adds extra information to the DNA that is not encoded in the nucleotide sequence (Jeltsch, 2002). Aberrant DNA methylation may relate to polymorphic variation in population, as well as aging and cancer (Katsutoshi et al., 2001). The methods for detecting DNA methylation are important in biological researches.

A variety of methods are used to detect DNA methylation: Southern blot (Bickle and Kruger, 1993), bisulfite genomic DNA sequencing (Frommer et al., 1992), restriction enzyme-PCR (Kane et al., 1997), methylation-specific PCR (Herman et al., 1996), electrochemistry (Hou et al., 2003a,b), etc. However, the present methods are laborious and time-consuming. Recently, DNA microarray has offered useful and high-throughput tools in studying the phenomenon of DNA methylation (Hou et al., 2004; Gitan et al., 2002; Adorján et al., 2002; Hatada et al., 2002; Balog et al., 2001; Hou et al., 2003a,b; Hacia, 1999). There are two different approaches to the DNA microarray fabrication. One approach is using a set of oligonucleotide probes arrayed on solid supports to discriminate the methylation patterns of one sample. The other approach, which could be used for screening a myriad of samples simultaneously, is that PCR products of bisulfite-treated genomic DNAs were immobilized on the slide (Zhou et al., 2006) and detected by a set of probes to identify the methylated cytosines for multiple samples.

However, the conventional method requires DNA samples should be converted, amplified and purified before being arrayed on the slide, which could be influenced by a myriad of factors in each process. First, researchers have to treat different DNA samples in separate reaction solutions, during which it consumes lots of labor time to repeat the identical multiple operations especially facing a large amount of samples. Also they have to endeavor to shift off the discrepancies of conversion caused by inevitable nonuniformity of reaction conditions and systems. Second, during the amplification process, it is necessary to optimize the PCR reaction condition, control the quality of products and likewise amplify different samples in separate solutions. At last, these PCR products should be purified in order to remove ions and proteins, and this process certainly adds the detection cost.

In this paper, we conceived a novel protocol to execute the bisulfite treatment of the immobilized DNAs directly

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on-chip and detect the methylation patterns of CpG status with fluorescence labeled probes by hybridization. In order to increase the amount of the immobilized DNAs, we adopted three-dimensional functionalized hydrophilic polyacrylamide gel films (Timofeev et al., 1996; Proudnikov et al., 1998; Rehman et al., 1999) to fabricate DNA microarray, and immobilized plasmid DNA by copolymerization between acrylamide monomers and acrylamide-modified DNA fragments. Another advantage of polyacrylamide gel is it has good chemical stability even if the slide was soaked in the bisulfite reaction mixtures for several hours. We have optimized the experimental conditions to increase the sensitivity and specificity. In contrast with conventional method, this method could convert a large amount of different samples through once bisulfite modification, ensure the uniformity of samples' pretreatment and directly detect the methylation status of multiple genes on-chip without amplification and purification. This novel method could be used as a convenient tool to detect the methylation status of the multiple genes for a large amount of samples in the future.

2. Experimental

DNA samples were treated and immobilized according to the route of Fig. 1. Firstly, DNA was restricted with enzymes, and linker bearing 5′-terminal acrylamide group was ligated to the sticky ends. Using universal acrylamide gel polymerization techniques, DNA was directly immobilized on the slide. Secondly, the bisulfite treatment was immediately carried out on-chip by soaking the chip in bisulfite reaction mixtures. Non-methylated cytosines of the immobilized DNA were converted into uracil and methylated cytosines remained unchanged. After bisulfite modification, we adopted four kinds of oligonucleotide probes for detecting the methylation patterns of CpG sites. Through electrophoresis, the methylation patterns of two adjacent CpG sites could be detected. The detailed experimental descriptions would be given in the following.

2.1. Preparation of DNA targets

The *pUC19* DNA (Fermentas) used in the studies was divided into two parts. One aliquot was treated by methylase *Sss*I (New England Biolabs) at 37 °C overnight in 50 μl of the reaction mixture as methylated samples. Another was not treated by methylase *Sss*I as unmethylated samples. The methylated samples generated in this way had 100% methylated cytosine in the test CpG sites, whereas the unmethylated samples had all unmethylated cytosine residues in the test CpG sites. Approximately 2 μg DNA was restricted with 50 U of *Hae*I (New England Biolabs) at 37 °C overnight in 50 μl of the reaction mixture. This enzyme could restrict the double-stranded circular DNA into small fragments. The mixture was then held 80 °C for 20 min to stop the reaction, and purified with QIAquick column (Qiagen). One microgram DNA was digested again with 25 U of *Eco*RI (New England Biolabs) at 37 °C overnight in 20 μl of the reaction mixture. The mixture was then held 65 °C for 20 min to denature the enzyme, and likewise purified with QIAquick column (Qiagen). DNA (1 μg) was mixed with 100 μM of unphosphorylated linkers H-17 and H-18 10 μl, respectively. The linker sequences were

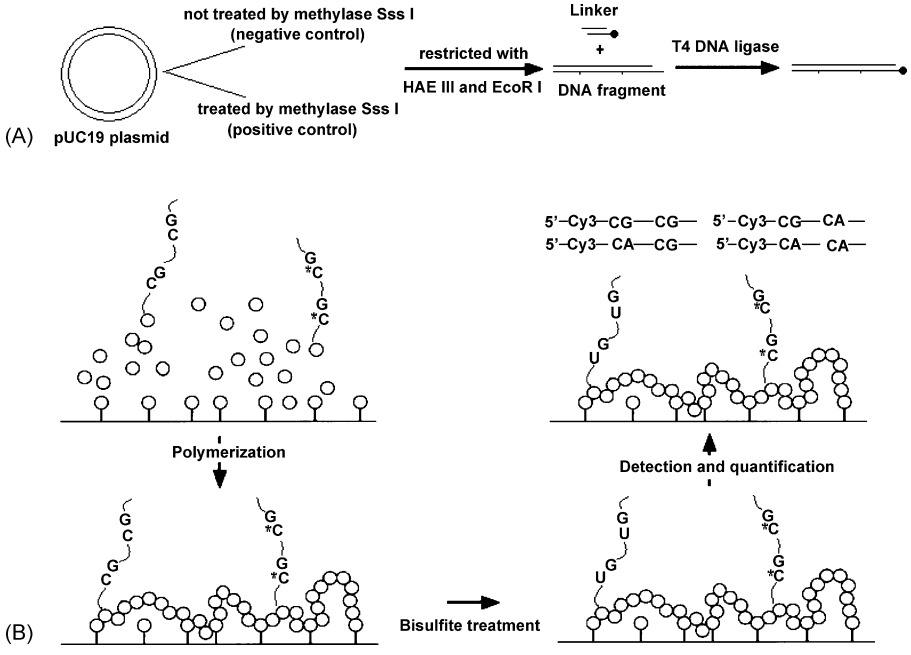

Fig. 1. (A) Plasmid DNA was divided into two parts. One aliquot was treated by methylase *Sss*I as methylated sample, the other was not treated by methylase *Sss*I as unmethylated sample. Then, DNA was restricted with *Hae*I and *Eco*RI, and linker bearing 5′-terminal acrylamide group was ligated to the sticky ends by T4 DNA ligase. (B) DNA fragments bearing 5′-terminal acrylamide modification efficiently copolymerize with acrylamide monomers on the acryl-modified slide then bisulfite modification on-chip to convert non-methylated cytosine, but not methylated, into uracil. At last, four kinds of different probes for hybridization to detect the methylation patterns of two adjacent CpG sites.