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High-throughput assay of DNA methylation based on methylation-specific primer and SAGE

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Abstract

Mapping of genomic DNA methylation is a dispensable part of functional genome. We have developed a novel method based on methylation-specific primer and serial analysis of gene expression, called MSP–SAGE with potential of high-throughput quantification of genomic DNA methylation. We used a 6-mer methylation-specific primer to extend the methylated CpG sequences other than non-methylated CpG sequences. The 17 bp tags contained methylated CpG sequence, which were obtained from extended methylation sequence by digestion of restriction endonuclease, and then the tags were concatenated and cloned for sequencing. We can identify the locations of methylation according to the sequences of tags and quantify the methylation status from the frequency of the tags. MSP–SAGE has a good linearity in a broad methylation range from 5% to 100% with good accuracy and high precision. The proof-of-principle study shows that MSP–SAGE is a reliable high-throughput assay for quantification of DNA methylation.

Keywords: DNA methylation; Quantification; High-throughput; Methylation-specific primer; SAGE

The primary function of DNA methylation at regulatory elements, notably promoters and enhancers, is to modulate gene expression. Remarkable evidences indicate that an individual's epigenetic profile can influence phenotype and susceptibility to various diseases including cancer [1], aging [2], and developmental disorders [3]. The mammalian genomic DNA methylation pattern varied in different tissues from different individuals in different times, termed tissue-specificity and inter-individual variation [4]. Therefore, the epigenetic map [5], namely the scanning of DNA methylation within whole genome, will be invaluable for the understanding of gene regulation and disease etiosis.

A variety of methods have been presented during the last few years for the analysis of CpGs methylation alleles using either digestion of DNA with methylation-sensitive restriction enzymes or bisulfite conversion, such as methylation-specific PCR (MSP) [6], combined bisulfite restriction analysis (COBRA) [7], and methylation-sensitive single nucleotide primer extension (Ms-SNuPE) [8]. Nonetheless, some of these methods such as MSP can only be classified into qualitative measurement and the other quantitative methods are often limited to one or several alleles. The so-called high-throughput assays such as methylation-specific oligonucleotide microarray (MSO) [9] and MALDI mass spectrometry [10] were restricted to limited regions with site-specific probes or site-specific primers. The project for DNA methylation mapping is much more complex than HGP, which cost human 13 years to be completed and therefore high-throughput quantification methods for mapping of genomic DNA methylation are needed urgently.

Bisulfite treatment of genomic DNA converts all non-methylated cytosines to uracils, whereas methylated cytosine is not changed under the reaction condition, then the methylation status can be determined from different

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sequences [11]. Serial analysis of gene expression (SAGE) is a brilliant idea for the scanning of gene expression and has some advantages than gene chips [12,13]. This high-throughput method can detect not only whether a gene expressed or not but also its expressing density, which ignited in us the idea to design a novel method based on methylation-specific primer and SAGE (MSP–SAGE) for high-throughput quantification of DNA methylation.

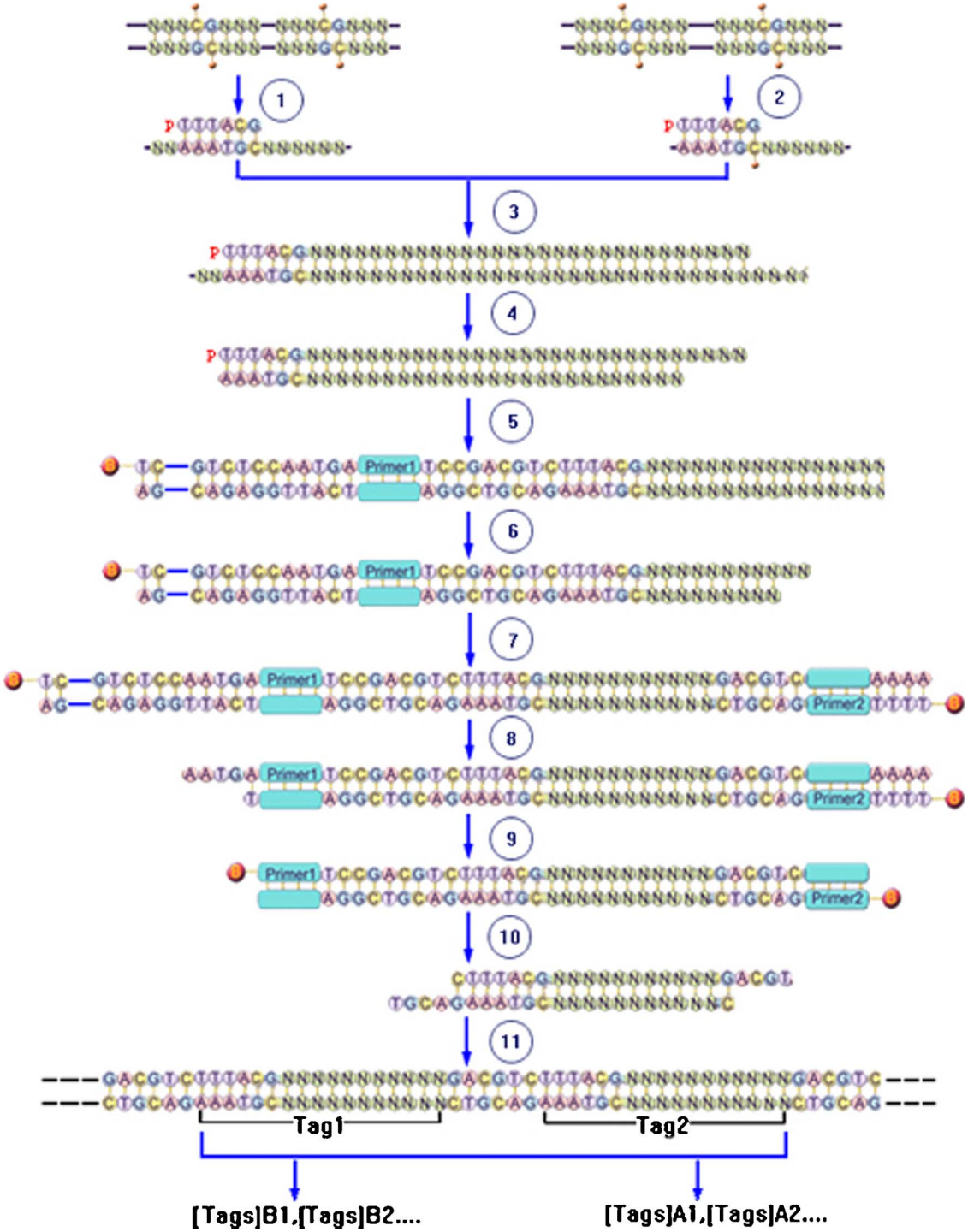

Fig. 1. The principle of MSP–SAGE. ① Annealing to the CG primers after denaturation of genomic DNA. ② Annealing to the CG primers after bisulfite treatment of genomic DNA. ③ Extension with Klenow DNA polymerase (performed in parallel from this step). ④ Annealing to common random primers and extended with *Escherichia coli* DNA polymerase I. ⑤ Ligated to linker 1 with T4 DNA ligase. ⑥ Cleaved with RE *Mme*I. ⑦ Ligated to linker 2 with T4 DNA ligase. ⑧ Cleaved with RE *Alw*26I. ⑨ PCR amplified with biotinylated primers. ⑩ Digested with RE *Aat*II. ⑪ Preparation of concatemers with T4 DNA ligase. The tags for samples with and without bisulfite treatment, termed [Tags]B and [Tags]A, can be identified from the sequencing results of following T clones, respectively. The density of methylation (%) at some allele can be calculated as $K \times [\text{Tag}]\text{B}/[\text{Tag}]\text{A} \times 100$.