A Novel One-Step Method for Rapid and Accurate DNA Methylation Quantification

Onyi Chima[1], Lam Nguyen[1], Michelle Rabot[1] & Xi Yu Jia[1]
[1]Zymo Research Corporation, Orange, CA

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The Beauty of Science is to Make Things Simple

Abstract:

DNA methylation plays a central role in widespread biological phenomena. Current methods used to evaluate DNA methylation such as Bisulfite Sequencing and Methylation Specific PCR present a number of problems as they are expensive, lengthy and involve multiple steps that increase chances of contamination. Therefore, we sought to develop a one-step system for rapid and accurate DNA methylation quantification. Here, we describe a simple method that exploits the specificity of Methylation Specific Restriction Enzymes (MSRE) to digest unmethylated CpG dinucleotides juxtaposed to real-time PCR for the selective amplification of methylated DNA. Thus, integration of the multiple steps of traditional procedures into one simple step decreases chances of contamination and allows for rapid and accurate DNA methylation percentage quantification. Our one-step MSRE-qPCR system selectively amplified methylated DNA and accurately measured methylation profiles of known DNA methylated standards. Methylation percentages of blind controls determined by the one-step system closely coincided with actual methylation percentages. This assay has proven to be a cost-effective assay for accurate region-specific methylation status quantification and will be a valuable tool in research and diagnostics

Introduction:

Epigenetic modifications are regarded as fundamental elements in gene expression regulation. DNA methylation, one such modification, plays crucial roles in widespread biological phenomena including host defense in bacteria and cell cycle regulation, gene imprinting, embryonic development and X-chromosome inactivation in mammals. Aberrant DNA methylation patterns in gene promoters are closely associated with perturbations in gene expression and have recently been indicated as leading cause of human cancers (Jones and Laird, 1999).

The field of epigenetics has grown exponentially in the scientific community as irregularities with gene expression due to abnormal DNA methylation is the leading cause in human cancer types. DNA methylation involves the chemical addition of a methyl group to the 5' carbon position on the cytosine pyrimidine ring. Most DNA methylation occurs within CpG islands which are commonly found in the promoter region of a gene. Thus, this form of post modification of DNA acts as communicative signal for activation or inactivation of certain gene expressions throughout all cell types.

Current methods used today to analyze DNA methylation status includes Me-DIP, HPLC, microarrays, and mass spectrometry, all of which uses relatively expensive or hard to obtain machinery that may inhibit further research work. The most common method for DNA methylation analysis involves bisulfite treatment, in which unmethylated cytosines are converted to uracil while methylated cytosines remain unchanged, followed by downstream amplification and sequencing. However, bisulfite treatment is labor intensive and can be difficult at times. A more simple and rapid analysis of DNA methylation at a region specific locus is digesting methylated DNA samples with methylation sensitive restriction enzymes (MSRE), followed by quantitative real time PCR. This one step process allows for a quick screening of percent methylation at specific CpG regions without the use of bisulfite treatment or any other costly machinery.

Concerting complete digestion and quantified amplification together into one step allows for a rapid analysis of differential methylation profile in DNA. Consequently, this method will provide an outlet for many clinical and academic lab researchers to quickly and precisely assess DNA methylation status in patients or in sensitive samples. By seamlessly combining MSRE digest and quantitative real-time PCR into one streamline process, DNA methylation status analysis at a gene specific region is faster and easier to handle with robust and clear results in a cost effective manner.

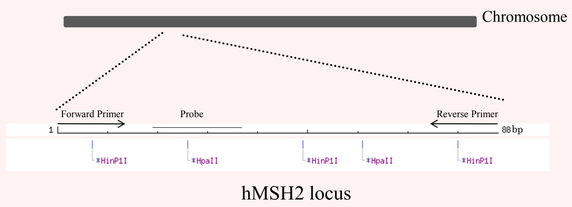
Methods:

The following reactions occur within the same tube:

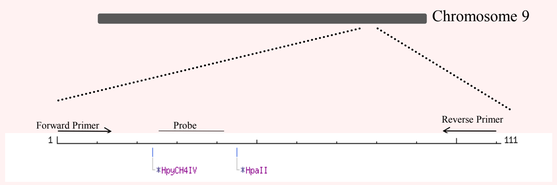
Methylated or Non-methylated DNA sample → Methylation Sensitive Restriction Enzyme Digest → qPCR

Percent methylation= $100^{2x(-\Delta Ct)}$

where ΔCt = Ct obtained from a sample incubated with an active MSRE reaction mixture minus the Ct obtained from a sample incubated in a reaction mixture without active MSRE



hMSH2 locus



DAPK1 locus

Human gDNA loci used in this study

Results:

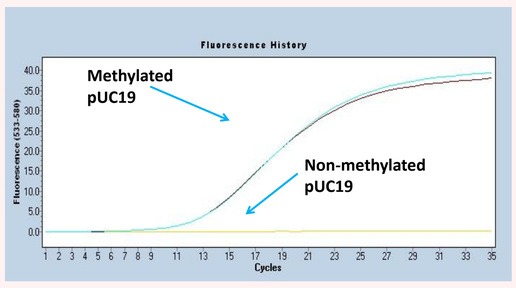
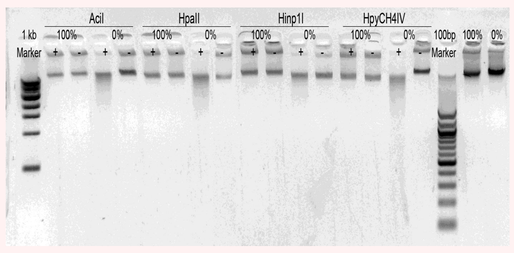


Figure 1. One-step MSRE-qPCR selectively amplifies methylated DNA. 5ng fully methylated and non-methylated pUC19 was subjected to one-step MSRE-qPCR. Methylated pUC19 (blue and brown curve) and non-methylated pUC19 (yellow line) were differentially amplified.

A.



B.

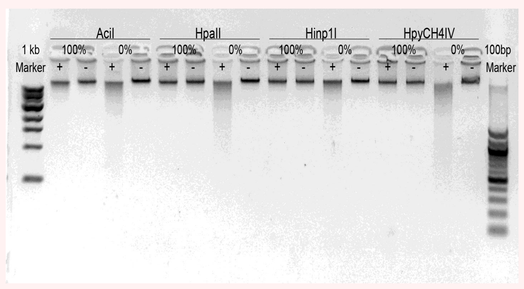


Figure 2. Digestion of fully and non-methylated DNA by One-step MSRE-qPCR master mix is comparable to optimal buffer conditions. Fully methylated and non-methylated human DNA were digested in either AciI, Hinp1I or HpyCH4IV or HpaII for 2hrs at 37ºC either under standard MSRE conditions (A) or using the one-step MSRE-qPCR master mix (B).

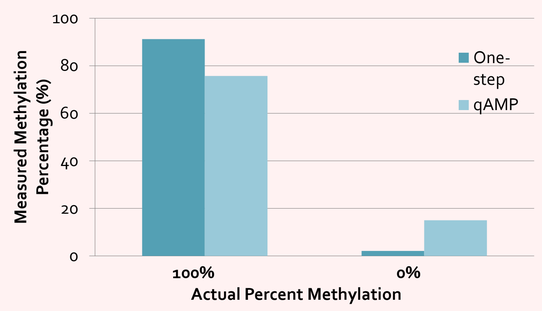


Figure 3. Comparison of qAMP methylation values to one-step MSRE-qPCR values. Fully methylated and non-methylated human gDNA was subjected to Hinp1I digestion and qPCR by employment of either qAMP or the one-step MSRE-qPCR method to ascertain the hMSH2 locus methylation status. Methylation percent values were calculated

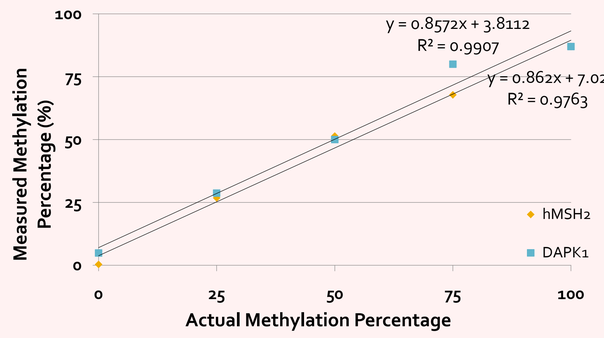


Figure 4. Methylation values of standards using one-step quantification method coincide with actual methylation values. Human DNA standards with known methylation percentages were MSRE-digested and amplified using the one-step MSRE-qPCR method. Methylation percent values of all CpGs in the hMSH2 locus (diamonds) and DAPK1 locus (squares) were calculated by applying ΔCt values to the established relationship.

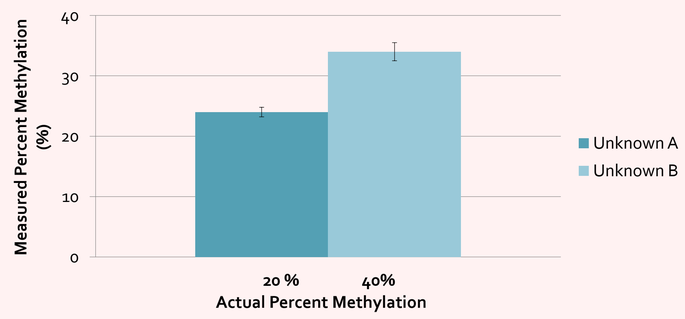


Figure 5. One-step methylation quantification method accurately determines methylation profiles of blind controls. Two blind controls prepared by mixing fully methylated or non-methylated human DNA with known methylation percentages were subjected to one-step MSRE qPCR method. ΔCt values were used to determine methylation values by use of the established relationship. Data represents standard deviation.

Conclusions:

One-step MSRE-qPCR system:
- unique single buffer system allows for simultaneous digestion by different MSREs
- juxtaposed to qPCR to reduce time and contamination
- selectively amplifies methylated DNA
- accurately quantifies percent methylation
- cost-effective, rapid assay will be a valuable tool in research and diagnostics

References:

Jones and Laird. Cancer Epigenetics Comes of Age.1999. *Nat Genet 1999; 21:163-7.*