**Sodium bisulfite can deaminate “convert” cytosine in DNA into uracil, but does not affect 5-methylcytosine. Bisulfite treatment of DNA is a prerequisite for DNA methylation analysis for many epigenetics-based studies involving methylation profiling and the quantification of methylation status. However, analytical procedures involving bisulfite treated DNA are often subject to variability due to DNA degradation, incomplete conversion, and/or low yields of DNA. We have systematically investigated the procedure of bisulfite treatment of DNA paying particular attention to the chemistries involved in the process and to conversion rates in an effort to limit variability between samples and to improve upon conventional methods. We found conventional bisulfite DNA conversion chemistries could be improved such that increased C to U conversion efficiencies could be obtained without the levels of DNA degradation typically resulting from incubation of reaction mixtures at high temperature and nonphysiological pH. Essential to this process was prohibiting the occurrence of “over-conversion” of 5-methylcytosines into uracil that can occur in some situations and reaction conditions. We found the bisulfite conversion process could be simplified and the variability between treatments kept to a minimum by coupling heat denaturation with the bisulfite conversion process and by using in-column desulfonation to clean and purify the converted DNA. This new method was found to yield an average of > 80% recovery of input DNA with < 99% C to U conversion. The method has been specifically designed to accommodate (in addition to purified DNA) biological fluids, cells, or tissue directly as the input material. This makes its application for FFPE and LCM-derived samples particularly well suited.**

**INTRODUCTION**

DNA methylation is a naturally occurring event in both prokaryotic and eukaryotic organisms. In prokaryotes DNA methylation provides a way to protect host DNA from digestion by restriction endonucleases that are designed to eliminate foreign DNA, and in higher eukaryotes DNA methylation functions in the regulation of gene expression (1). It has been demonstrated that aberrant DNA methylation is a widespread phenomenon in cancer and may be among the earliest changes to occur during oncogenesis (2). DNA methylation has also been shown to play a central role in gene imprinting, embryonic development, X-chromosome gene silencing, and cell cycle regulation. In many plants and animals, DNA methylation consists of the addition of a methyl group to the fifth carbon position of the cytosine pyrimidine ring via a methyltransferase enzyme (3). The majority of DNA methylation in mammals occurs in 5-CpG-3 dinucleotides, but other methylation patterns do exist. In fact, about 80 percent of all 5-CpG-3 dinucleotides in mammalian genomes are found to be methylated, whereas the majority of the twenty percent that remain unmethylated are within promoters or in the first exons of genes.

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, as well as many other important aspects of biology. To date, a number of methods have been developed to detect/diagnose DNA methylation including: high-performance capillary electrophoresis (4) and methylation-specific polymerase chain reaction (PCR) assays (5). However, the most common technique used today remains the bisulfite conversion method (6). This technique involves treating methylated DNA with bisulfite, which converts unmethylated cytosines into uracil. Methylated cytosines remain unchanged.

**RESULTS AND METHODS**

**Figure 1: Methylation Profiling by Bisulfite Sequencing.** A simple procedure for methylation detection is bisulfite sequencing. DNA bisulfite conversion is followed by PCR amplification and subsequent display of cytosine methylation pattern. Different methods are often associated with incomplete conversions and over conversions.

**Figure 2: Bisulfite Conversion of Cytosine to Uracil.**

<table>
<thead>
<tr>
<th>Step</th>
<th>Reaction</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sodium bisulfite deamination</td>
<td>Direct reaction between cytosine and bisulfite yields uracil</td>
</tr>
<tr>
<td>2</td>
<td>Hydrolytic deamination</td>
<td>Uracil is converted to cytosine by acid catalyzed cleavage</td>
</tr>
<tr>
<td>3</td>
<td>Abolish deamination</td>
<td>Uracil is converted back to cytosine by alkaline catalyzed cleavage</td>
</tr>
</tbody>
</table>

**Figure 3: High Efficiency Recovery Of Input DNA.** The indicated amounts of genomic DNA were processed using the EZ DNA Methylation-Direct™ (Direct), EZ DNA Methylation-Gold™ (Gold), or EZ DNA Methylation-EZM™ (EZM) Kit. The recovered DNA was quantified in quadruplicate sets. Error bars represent ± 1 standard deviation. All kits performed very well, with average recoveries above 70% at all DNA inputs. While the Gold and EZM kits began to decline in recovery below 500 ng of input, the Direct kit maintained greater than 80% recovery across the input spectrum from 125 to 1000 ng.

**Figure 4: Improve the Conversion reaction kinetics.** A plasmid universally methylated in all CpG dinucleotides was converted using either the conventional method or improved method. A time course was conducted to evaluate the conversion efficiency as the reaction progressed. All samples were processed, amplified, and sequenced. Conversion errors were tabulated for each group of sequences and progressive. All samples were processed, amplified, and sequenced.

**Figure 5: Simple method to analyze Bisulfite Converted DNA Using Agarose Gel.**

- **No chill**
- **Chilled**

**Figure 6: Sensitivity of Detection Using New Method.** A suspension of human primary fibroblasts was serially diluted 1:10 in digestion buffer and proteinase K. These dilutions were then digested at 50°C for 20 minutes. 20 µl of each digestion were converted and processed using the Direct kit. PCR was conducted and results were analyzed on 2% agarose gel. The results show that the method is sensitive enough to detect as few as 10 cells.

**REFERENCES**


**SELECTED CITATIONS**