Direct Bisulfite Conversion from Archived Tumor Samples for Methylation Detection

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ABSTRACT

DNA methylation is an important epigenetic mechanism of transcriptional control. It plays an essential role in maintaining cellular function, and changes in methylation patterns may contribute to the development of cancer. Aberrant methylation of DNA is frequently found in tumor cells. Formalin-fixed and paraffin-embedded (FFPE) tissue sections are useful for routine histopathological diagnosis, but they have increased in popularity for molecular studies of genome using molecular biological techniques such as PCR, qPCR, sequencing, genotyping, etc. A major limitation of FFPE samples is the significant degradation of the nucleic acids recovered from fixed tissues especially for the aged, long-term archived samples. Since FFPE samples make the huge collection of clinical tissue banks, more and more efforts have been spent in recovering the high quality of DNA from these specimens. Although the number of epigenetic cancer studies continues to grow, the wealth of FFPE samples available remains largely untapped. The value of FFPE samples to studies of the epigenome and the role of DNA methylation in numerous oncological processes and disease, including cancer, has been recognized more and more. We developed a new bisulfite conversion method that not only eliminates the deparaffinization but also eliminates the genomic DNA purification procedure. In our procedure, FFPE samples were directly treated with bisulfite and then amplified by bisulfite conversion and denaturation procedures. The whole process can be completed within 2 hours. Even for long-term archived samples, short length of DNA fragments can be recovered after the conversion of genomic DNA were produced which was used for methylation detection at single CpG level in this work. Clinical archived breast cancer and adjacent normal tissues as well as paired lung cancer and colon cancer samples were directly bisulfite converted. Breast cancer, colon cancer as well as lung cancer related promoter regions APC, BCL2, PTEN, H3F3B and SCGB5A1 were compared for methylation changes.

INTRODUCTION

A few methods have been widely used for methylation detection nowadays. Among the methods available, bisulfite conversion remains to be the most commonly used technique as it can provide single CpG site methylation detection. The bisulfite conversion involves treating DNA with bisulfite to convert unmethylated cytosine into uracil, while methylated cytosine remains unchanged. Upon conversion, methylation profiling can be determined by sequencing or real time PCR or high resolution melting (HRM). Currently, most bisulfite conversion requires isolation of gDNA from FFPE samples. All protocols require deparaffinization of the specimen prior to DNA extraction. Deparaffinization is usually accomplished with 2 to 3 incubations in xylene and can be a nuisance, particularly if there are more than just a few specimens to deal with. New method was developed here to facilitate direct bisulfite conversion from FFPE samples without pre-deparaffinization.

MATERIALS and METHODS

In this study, we focused on three most common cancers in America: breast, colon and lung. Matched pairs of primary tumor and normal adjacent tissue for all three cancer types were obtained from Biochain (Hayward, CA). Paired tumor and normal adjacent archived tissue samples (FFPE, archived for 5-9 years) were cut into 10...