

Introduction

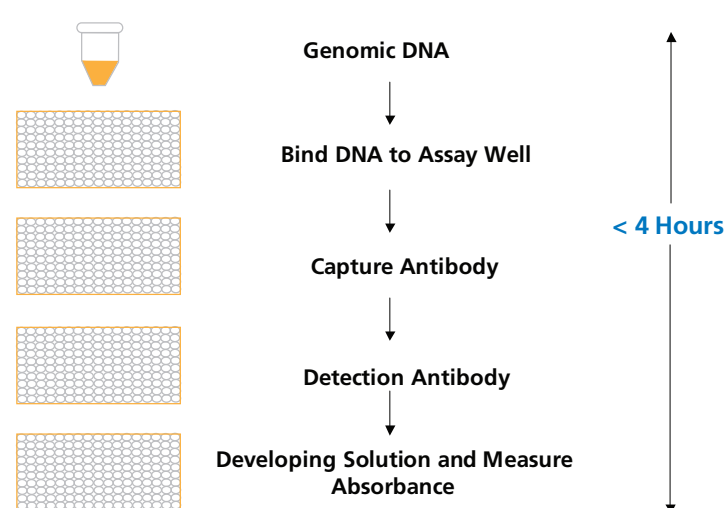
It has been well demonstrated that DNA methylation plays an important role in the regulation of gene expression. Shifts away from normal global DNA methylation levels have been observed in various cancers, neurological disorders, autoimmune diseases, and aging.

Several methods exist to measure global methylation levels but each have disadvantages including the cost of required equipment (i.e. mass spectrometry methods), lengthy protocols (i.e. enzymatic degradation and analysis), low sensitivity (i.e. immunohistostaining) and/or radioactive components. We have developed a new method, similar to a sandwich ELISA, which allows fast measurement of overall DNA methylation status.

Materials and Methods

Matched pairs of tumor tissue genomic DNA were obtained from Biochain Institute (Cat. Nos. D8235152-PP-10 and D8235149-PP-10, Hayward, CA). CHO DG44 cells were purchased from Invitrogen Corporation (Cat. No. 12609-012, Carlsbad, CA) and cultured according to the product manual. Liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) was performed as described by Song et al., Analytical Chemistry 2005. Global methylation was measured as described in the technical bulletin of Sigma's Imprint® Methylated DNA Quantification Kit (Cat. No. MDQ1). Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich (St. Louis, MO).

Sigma Imprint Methylated DNA Quantification Protocol



Results

Discrimination Between Methylated and Unmethylated DNA

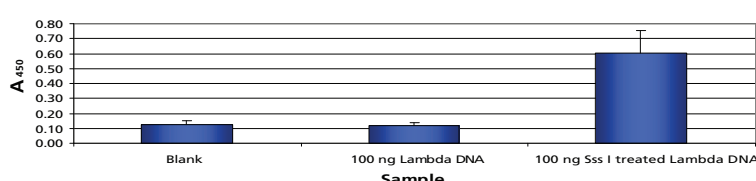


Figure 1: Unmethylated Lambda DNA was treated with Sss I Methylase. 100 ng of untreated and treated Lambda DNA was analyzed with the Imprint Methylated DNA Quantification kit. The untreated sample was comparable to the blank indicating no methylation present. The treated sample had a 6-fold increase in signal indicating that methylation occurred due to treatment with methylase.

Specific Detection of Methylated DNA Diluted with Unmethylated DNA

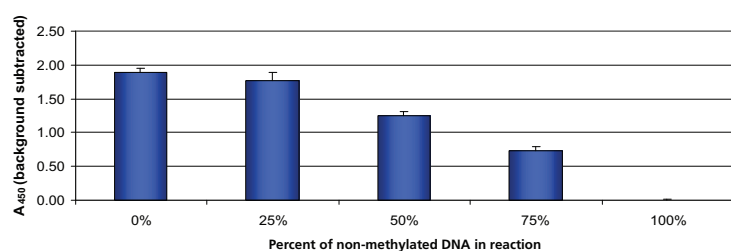


Figure 2: Fully methylated human genomic DNA was subjected to whole genome amplification. The resulting completely unmethylated product was mixed with fully methylated DNA. Global methylation of 100 ng of the various methylated/unmethylated DNA mixtures was measured. As the amount of unmethylated DNA increases, the A₄₅₀ signal strength decreases in proportion to the amount of unmethylated DNA in the sample.

Differential Detection of Two Methylated DNA Samples

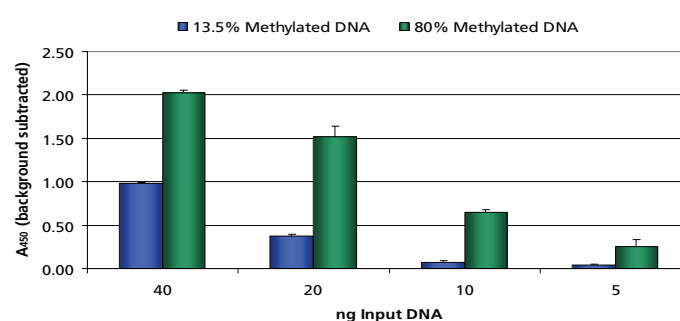
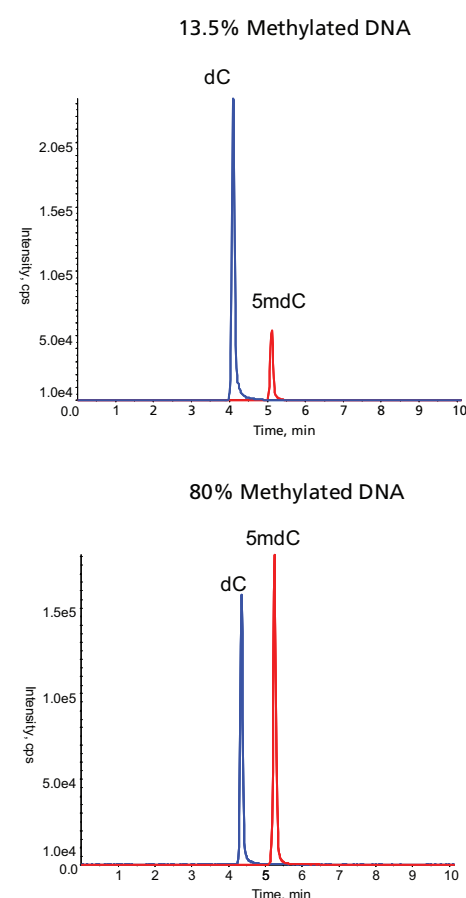


Figure 3: Two lots of DNA were treated with Sss I Methylase. Both lots were analyzed by LC-MS/MS to determine percent global methylation. Varying amounts of DNA were also analyzed by the Imprint Methylated DNA Quantification kit. As demonstrated by the graph, the difference in methylation states can easily be detected and the signal generated is proportional to the amount of DNA used in the procedure.

Detection of Azacytidine DNA Demethylation

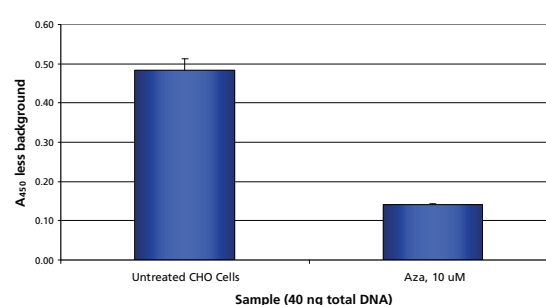


Figure 4: Azacytidine, a known demethylating agent, was added directly to the media of CHO DG44 cells. The cells were harvested after 48 hours. Global methylation was measured on the purified CHO DG44 DNA. 40 ng of DNA from treated and non treated cultures was analyzed. As expected, the azacytidine treated cells were less methylated than the untreated cells.

Global DNA Methylation Measurement of Matched Pair Lung Tissue DNA

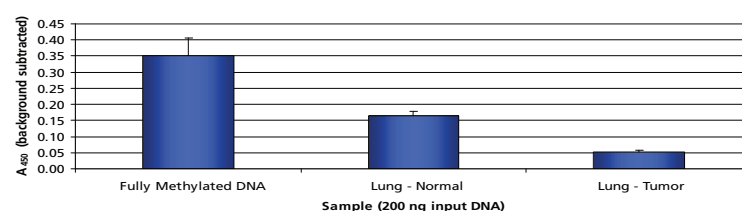


Figure 5: DNA was isolated from bronchioalveolar carcinoma and adjacent normal lung tissue from a 72 year old male. Global DNA methylation was measured for both samples and compared to a fully methylated human genomic DNA control. As expected (Mao L, Cancer 2008), the adjacent normal lung tissue was less methylated than the fully methylated control and the tumor DNA was hypomethylated when compared to normal lung tissue.

Global DNA Methylation Measurement of Matched Pair Liver Tissue DNA

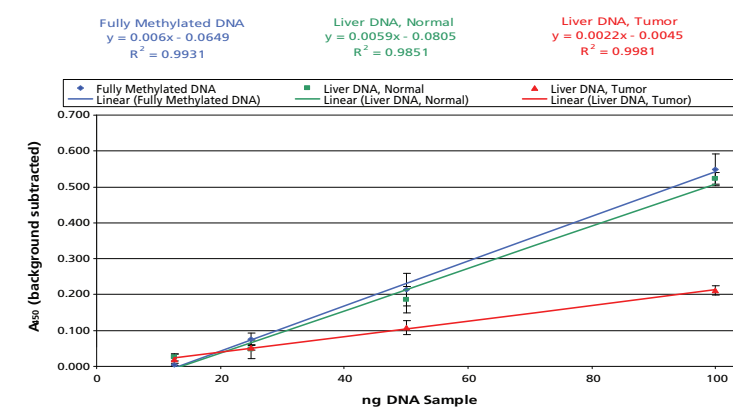


Figure 6: DNA was isolated from hepatocellular carcinoma and adjacent normal liver tissue from a 54 year old male. Global DNA methylation was measured for both samples and compared to a fully methylated human genomic DNA control. The normal liver DNA was slightly less methylated than the control. Previous publications (Calvisi et al. J Clin Invest 2007) indicate that hepatocellular carcinoma is hypomethylated. Our data demonstrates that tumor liver DNA is hypomethylated in this comparison.

Discussion/Conclusion

Until now, measurement of global DNA methylation could be cumbersome and oftentimes cost prohibitive. We have demonstrated that our ELISA-based method, the Imprint Methylated DNA Quantification kit, simplifies the process and provides results in about half a day in a high throughput format.

Our method easily distinguishes between methylated and unmethylated DNA. Methylated DNA can be detected in a mixed population of methylated and unmethylated DNA. The signal generated by this method is proportional to the amount of methylated DNA in the sample.

DNA from biological systems such as cultured cells, normal tissue and tumors have also been analyzed. The global methylation levels determined with our method correspond with data generated by other methodologies.

Future Work

While the Imprint Methylation DNA Quantification kit is useful in distinguishing relative levels of global DNA methylation, LC-MS is considered the gold standard for making absolute measurements. Work is underway to validate our ELISA-based method against LC-MS. Samples to be analyzed include Sss I methylated DNA, normal human genomic DNA, tumor DNA, and DNA from various cell lines. The data generated from these comparisons is expected to provide a correlation coefficient between LC-MS analysis and our method.

Additionally, experiments are underway to study the effect of the miRNA 29 family on global DNA methylation levels in the lung cancer cell line A549 to demonstrate the utility of the Imprint Methylated DNA Quantification kit.

References

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Acknowledgments

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