

TECHNOLOGY REVIEW

METHYLCODE™ BISULFITE CONVERSION KIT

Detect and analyze DNA methylation patterns accurately and efficiently.

DNA methylation is an epigenetic event that can alter gene expression. In humans, approximately 1% of DNA bases undergo methylation and about 10–80% of 5'-CpG-3' dinucleotides are methylated. Aberrant hypermethylation of CpG islands can occur and has been linked to changes in transcription and gene expression. These events have been shown to play a central role in cancer, gene imprinting, embryonic development, and many other biological functions.^{1–3} The MethylCode™ Bisulfite Conversion Kit provides a simple, rapid method for accurate and efficient detection and analysis of methylation patterns.

Streamlined MethylCode™ method produces better results. The most commonly used technique for detecting methylation in specific regions of DNA is the sodium bisulfite conversion method. In this method, DNA is denatured and treated with sodium bisulfite, causing unmethylated cytosines to convert to uracils while methylated cytosines remain unchanged.⁴ Converted DNA can be PCR amplified and analyzed by DNA sequencing or restriction endonuclease digestion and the methylation patterns determined by comparison to untreated DNA (Figure 1).

The MethylCode™ Bisulfite Conversion Kit improves upon this approach, reducing the number of steps required and the overall time to achieve results while increasing the conversion efficiency. The streamlined

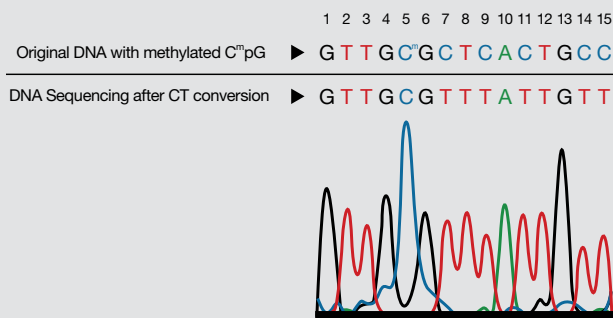


Figure 1—Complete conversion of unmethylated cytosines into uracils after bisulfite treatment. DNA with methylated CpG (nucleotide position 5) was processed using the MethylCode™ Bisulfite Conversion Kit. The recovered DNA was amplified by PCR, cloned, and sequenced. Following bisulfite treatment, the methylated cytosine at position 5 remained intact while the unmethylated cytosines (positions 7, 9, 11, 14, and 15) were converted into uracils and detected as thymines following PCR.

protocol (Figure 2) integrates the DNA denaturation and bisulfite conversion processes into one convenient step by replacing chemical denaturation using sodium hydroxide with simple temperature denaturation. In addition, cumbersome postconversion DNA precipitation steps are eliminated through use of an innovative in-column desulfonation technology. This minimizes template degradation and DNA loss so that as little as 500 pg of starting material is required. Recovered DNA is ready for PCR amplification and for use in a number of downstream analysis applications, including restriction endonuclease digestion, sequencing, and microarrays. Complete conversion of unmethylated cytosines as well as DNA purification can be achieved in less than 3 hours, a considerable savings compared with traditional methods that require overnight incubation.

Proven performance. The MethylCode™ Bisulfite Conversion Kit can be used to study DNA methylation, its effect on gene regulation, and the role it may play in particular disease states. To demonstrate, the methylation patterns of estrogen receptor alpha (ERα) were examined in

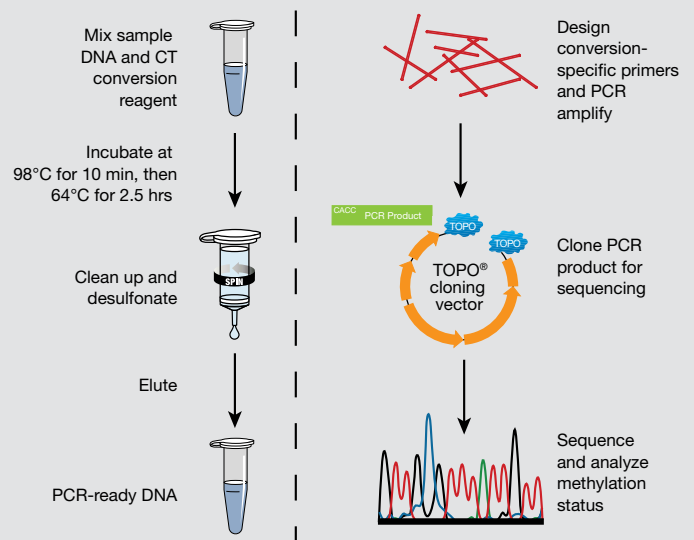


Figure 2—Streamlined MethylCode™ bisulfite conversion protocol is complete in just three hours. Sample DNA is temperature denatured and converted, and the converted DNA is precipitated using an in-column desulfonation technology, then eluted. The DNA can then be PCR amplified, cloned, sequenced, and analyzed for methylation patterns.

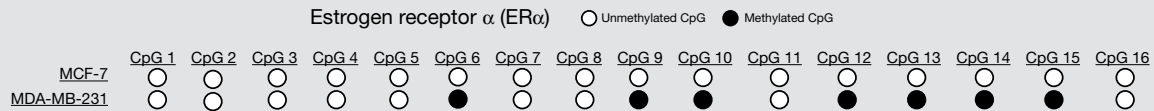


Figure 3—Methylation patterns of ER α as analyzed using the MethylCode™ Kit. Genomic DNA from MCF-7 and MDA-MB-231 cell lines was isolated and treated with the MethylCode™ Bisulfite Conversion Kit. Bisulfite-converted DNA was then used as template in PCR to amplify a portion on the estrogen receptor alpha (ER α) gene. Next, the PCR product was cloned into a sequencing vector and sequenced. Finally, the sequences were analyzed to show a differential methylation pattern of the ER α gene between the MDA-MB-231 and MCF-7 cell lines. Locus = X03635 6450 bp mRNA linear PRI 11-JUN-2003; Definition = *Homo sapiens* mRNA for estrogen receptor; Accession = X03635 M11457; Version = X03635.1 GI:31233.

early- (MCF-7) and late-stage (MDA-MB-231) breast cancer cells. ER α is a nuclear hormone receptor whose down-regulation or mutation has been shown to be involved in a variety of diseases, including breast cancer, type 2 diabetes, and cardiovascular disease.⁵⁻⁷

After purification, gDNA from both cell types was bisulfite converted using the MethylCode™ Bisulfite Conversion Kit. The gene fragment of interest was then PCR amplified from each purified DNA sample using bisulfite conversion–specific primers, cloned, and sequenced. Sequencing results from five clones per cell line were compared and analyzed for differential methylation patterns. As shown in Figure 3, there is detectable hypermethylation of ER α in the late-stage MDA-MB-231 cells compared to the early-stage MCF-7 cells. These results agree with previously published research,⁸ indicating that these changes in methylation may play an important role in tumor progression. These results demonstrate the accuracy of the MethylCode™ Bisulfite Conversion Kit in methylation profiling studies.

Powerful methylation pattern profiling. The MethylCode™ Bisulfite Conversion Kit provides a fast, efficient, and accurate method for analyzing methylation patterns and furthering the understanding of complex diseases. To learn more about the MethylCode™ Bisulfite Conversion Kit, visit www.invitrogen.com/methylcode or contact us at locations worldwide (page 55).

REFERENCES

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Product	Quantity	Cat. no.
MethylCode™ Bisulfite Conversion Kit	50 rxns	MECOV-50
MethylCode™ Bisulfite Conversion Service	NA	MECOV-SVC

NA = not applicable.