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Universal Methylated DNA Standard and Control Primers

Catalog No. D5010

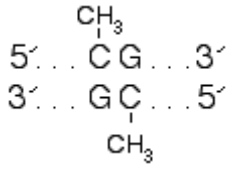
For Research Use Only

Description

The **Universal Methylated DNA Standard** includes enzymatically methylated DNA together with a specially-designed primer set to be used in conjunction with Zymo Research's **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™**, and **EZ DNA Methylation-Direct™ Kits** to assess the efficiency of bisulfite-mediated conversion of DNA. Central to this, is the pUC19 DNA that was isolated from a methylation-negative strain of bacteria (*dam*$^-$, *dcm*$^-$) prior to its enzymatic modification with *Sss* I methylase (EC 2.1.1.73). The DNA is methylated at cytosine positions comprising CG dinucleotides. The primer set herein has been designed to amplify a fragment of the supplied pUC19 DNA following bisulfite treatment. The methylated cytosines comprising CG dinucleotides remain unconverted following bisulfite treatment, whereas nonmethylated cytosines are converted into uracil and detected as thymine following PCR. The supplied methylated pUC19 DNA was linearized at position 2177 using *Sca* I endonuclease.

I. Universal Methylated DNA Standard, 20 µl. This standard contains pUC19 DNA, which contains methylated cytosines (C^5) at all CG dinucleotide positions.

Methylation Site:



Concentration: 5 pg/µl of universal methylated pUC19 DNA and 250 ng/µl of salmon sperm DNA as carrier in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH8.0).

II. Control Primers

Two primers are supplied at a concentration of 20 µM in 20 µl TE buffer.

Methyl Primer I (sense) containing *Xho* I site (in **bold**) for cloning.
5´-CT**CTCGAG**AAAATATCGTATTAGGCGTTATTCGTT-3´

Methyl Primer II (antisense) containing *Bam* H I site (in **bold**) for cloning.
5´-CG**GGATCC**AACCGCCTCTCCCCGCGCGTTAACCG-3´

The expected PCR amplicon is **466 bp**, corresponding to nucleotide **221-670** of pUC19 sequence plus, and an additional 16 bp added from the primers for cloning purposes.

Procedure

This control set can be used in conjunction with both the **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™**, and **EZ DNA Methylation-Direct™ Kit** procedures. The supplied primers can be used for sequencing directly.

Use 1 µl of supplied control template for each bisulfite conversion reaction. Follow the instructions in the kit for the bisulfite conversion process.

1. PCR setup

The following reaction is designed for total 25 µl reaction volume.

1 µl	Control Primer I
1 µl	Control Primer II
1 µl	of recovered DNA from the bisulfite reaction
2 µl	dNTP, 2.5 mM of each nucleotide
2.5 µl	10X PCR reaction Buffer
0.25 µl	Taq polymerase
17.25 µl	water

2. PCR Cycle

A. 95°C, 60 seconds
B. 94.5°C, 30 seconds
C. 59°C, 30 seconds
D. 72°C, 60 seconds
E. 72°C, 7 minutes

Cycle B to D 30 times. The PCR amplicon amplified by this primer set can be used directly for T/A cloning or can be cloned directly into a vector of choice containing *Xho* I and *Bam* H I sites.

Appendix

Original sequence of the PCR amplified pUC19 DNA fragment (sense strand 5' to 3'). All cytosines (underlined) in CG dinucleotides are methylated. Numbers correlate to the nucleotides from pUC19 DNA.

```
201 ---------- ---------- aaaatacCGc atcaggCGcc attCGccatt caggctgCGc
261 aactgttggg aagggCGatC GgtgCGggcc tcttCGctat taCGccagct ggCGaaaggg
321 ggatgtgctg caaggCGatt aagttgggta aCGccagggt tttcccagtc aCGaCGttgt
381 aaaaCGaCGg ccagtgaatt CGagctCGgt accCGgggat cctctagagt CGacctgcag
441 gcatgcaagc ttggCGtaat catggtcata gctgtttcct gtgtgaaatt gttatcCGct
501 cacaattcca cacaacataC GagcCGgaag cataaagtgt aaagcctggg gtgcctaatg
561 agtgagctaa ctcacattaa ttgCGttgCG ctcactgccC Gctttccagt CGggaaacct
621 gtCGtgccag ctgcattaat gaatCGgcca aCGCGCGggg agaggCGgtt
```

Expected Sequence of PCR amplified product after bisulfite conversion (sense strand 5' to 3').

```
201 ---------- ---------- aaaatatCGt attaggCGtt attCGttatt taggttgCGt
261 aattgttggg aagggCGatC GgtgCGggtt ttttCGttat taCGttagtt ggCGaaaggg
321 ggatgtgttg taaggCGatt aagttgggta aCGttagggt ttttttagtt aCGaCGttgt
381 aaaaCGaCGg ttagtgaatt CGagttCGgt attCGgggat tttttagagt CGatttgtag
441 gtatgtaagt ttggCGtaat tatggttata gttgtttttt gtgtgaaatt gttattCGtt
501 tataatttta tataatataC GagtCGgaag tataaagtgt aaagtttggg gtgtttaatg
561 agtgagttaa tttatattaa ttgCGttgCG tttattgttC Gttttttagt CGggaaattt
621 gtCGtgttag ttgtattaat gaatCGgtta aCGCGCGggg agaggCGgtt
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