ZYMO RESEARCH

Epigenetics
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Methylated & Non-methylated pUC19 DNA Set

Standards for DNA methylation analysis workflows

Highlights

- Ideal positive and negative controls for methylation detection assays.
- *In situ* spike-in controls for monitoring bisulfite conversion efficiency in Next-Generation Sequencing experiments.
- Provided pUC19MN primer pair allows for convenient assay quality control.

Catalog Numbers:
D5017

Scan with your smart-phone camera to view the online protocol/video.

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Product Contents

Methylated & Non-methylated pUC19 DNA Set	D5017	Storage Temp.
Methylated pUC19 DNA	20 ng/20 µl	-20ºC
Non-methylated pUC19 DNA	20 ng/20 µl	-20ºC
pUC19MN Primers	20 µl	-20ºC

Specifications

Methylated pUC19 DNA

- **Source –** pUC19 plasmid purified from Dam⁻, Dcm⁻ *E. coli* [enzymatically methylated by M.SssI Methyltransferase (EC 2.1.1.37)].
- **Concentration –** 1 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Non-methylated pUC19 DNA

- **Source –** pUC19 plasmid purified from Dam⁻, Dcm⁻ *E. coli*.
- **Concentration –** 1 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

pUC19MN Primers

- **Concentration –** 20 µM each primer in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).
- **Primer sequences –**

  pUC19MN Forward Primer:

  5′ – GGTTATAGTTGTTTTTTGTGTGAAATTGTTATT – 3′

  pUC19MN Reverse Primer:

  5′ – CTAACCTTTTACTCACATATTCTTTCCTAC – 3′

Product Description

The **Methylated & Non-methylated pUC19 DNA Set** consists of two control DNAs (methylated and non-methylated) along with a set of specifically designed primers that can be used in conjunction with the **EZ DNA Methylation™**, **EZ DNA Methylation-Gold™**, **EZ DNA Methylation-Direct™**, and **EZ DNA Methylation-Lightning™** kits from Zymo Research to assess the efficiency of bisulfite-mediated conversion of DNA. These plasmids can be used in conjunction with genomic DNA to provide internal controls to assess bisulfite conversion efficiency or to produce known mixtures of methylated and non-methylated DNA for assay calibration.

The **Non-Methylated pUC19 DNA** is pUC19 DNA that was isolated from a methylation-negative strain of *E. coli* (Dam⁻, Dcm⁻) and can be used as a negative control for DNA methylation analysis. The **Methylated pUC19 DNA** is pUC19 DNA that has been isolated from the same strain and has been enzymatically methylated at all cytosine positions comprising CG dinucleotides by M.SssI methyltransferase[1] (EC 2.1.1.37; Figure 1) and can be used as a positive control for DNA methylation analysis.

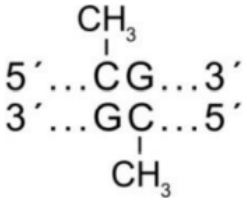

Figure 1. M.SssI methyltransferase methylates all cytosine residues in the double stranded CpG context.

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 in the **Methylated pUC19 DNA** remain unconverted following bisulfite treatment, whereas nonmethylated cytosines are converted into uracil and detected as thymine after PCR. The supplied pUC19 DNA has been linearized at position 2177 using ScaI endonuclease.

[1]Nur et al. J. Bacteriol. 164: 19-24 (1985).

Recommended Usage

The **Methylated and Non-methylated pUC19 DNA Set** can be used in a variety of methylation analysis applications including bisulfite and methylation-specific PCR, *in situ* spike-in controls, methylation sensitive high resolution melt analysis, methylation arrays, methylated DNA immunoprecipitation (MeDIP), library preparation, and more.

Protocol

For best results, it's important to ensure the DNA is completely homogenous and fully in solution before quantification and usage. The following steps are recommended before quantification and usage[1]:

1. Bring the standards to room temperature.
2. Mix the standards well, briefly spin down for 5-10 seconds.
3. Repeat Step 2 twice, for three times total.
4. Proceed with quantification or usage.

Bisulfite Conversion: For most applications 5-50 pg of plasmid may be used as an internal spike-in control for bisulfite conversion reactions containing 250 ng to 2 µg of genomic DNA. Refer to the kit specifications for setup of the bisulfite conversion reaction.

Bisulfite PCR Setup: The following is designed for a 25 µl reaction.

Component	Volume	Final Concentration
pUC19MN Primers[2]	Variable	0.2 to 0.8 µM each
Bisulfite-converted DNA[3]	1 µl	Up to 0.25 pg/µl
10 mM dNTP mix	0.5 µl	0.2 mM each dNTP
Standard PCR Buffer	Variable	1X
$MgCl_2$ or $MgSO_4$	Variable	1-4 mM, if needed
ZymoTaq™ DNA Polymerase[4]	Variable	1-2 units
Nuclease Free Water	Bring reaction to 25 µl	N/A

Recommended Thermocycler Conditions:

A. 95 °C, 10 minutes
B. 95 °C, 30 seconds
C. 57 °C, 30 to 60 seconds
D. 72 °C, 60 seconds
E. Repeat steps B through D an additional 29 to 39 times depending on the polymerase used.
F. 72 °C, 7 minutes
G. 4 °C

[1]Standards are quantified using NanoDrop® measurements. If using other methods, variation may be observed.
[2]Alternatively, you may substitute primers of your choice.
[3]Remember to bisulfite-treat the DNA prior to performing PCR.
[4]We recommend using **ZymoTaq™ DNA Polymerase** or other hot-start DNA polymerases for amplification of bisulfite-treated DNA.

Appendix

pUC19MN Bisulfite PCR

The expected PCR amplicon for the Methylated & Non-methylated pUC19 DNA is 362 bp, corresponding to nucleotide positions 464 to 825 of the pUC19 sequence, including the regions (italicized) that hybridize to the primers.

Original sequence of pUC19 for bisulfite treatment and PCR amplification (sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capital letters) are methylated enzymatically by M.SssI methyltransferase:

```
      5′ – ggtcatagct gtttcctgtg tgaaattgtt atcCGctcac
aattccacac aacataCGag cCGgaagcat aaagtgtaaa gcctggggtg
cctaatgagt gagctaactc acattaattg CGttgCGctc actgccCGct
ttccagtCGg gaaacctgtC Gtgccagctg cattaatgaa tCGgccaaCG
CGCGgggaga ggCGgtttgC GtattgggCG ctcttcCGct tcctCGctca
ctgactCGct gCGctCGgtC GttCGgctgC GgCGagCGgt atcagctcac
tcaaaggCGg taataCGgtt atccacagaa tcaggggata aCGcaggaaa
gaacatgtga gcaaaaggcc ag – 3′
```

Expected sequence of the above PCR amplicon following bisulfite treatment:

Methylated pUC19 DNA: Below is the expected sequence for the Methylated pUC19 DNA after bisulfite conversion and PCR (sense strand). Methylated cytosines in the CpG dinucleotide context remain unconverted following bisulfite treatment, whereas non-methylated cytosines, or cytosines not in the CpG context, are converted to uracil and detected as thymine after PCR.

```
      5′ – ggttatagtt gttttttgtg tgaaattgtt attCGtttat
aattttatat aatataCGag tCGgaagtat aaagtgtaaa gtttggggtg
tttaatgagt gagttaattt atattaattg CGttgCGttt attgttCGtt
ttttagtCGg gaaatttgtC Gtgttagttg tattaatgaa tCGgttaaCG
CGCGgggaga ggCGgtttgC GtattgggCG ttttttCGtt ttttCGttta
ttgattCGtt gCGttCGgtC GttCGgttgC GgCGagCGgt attagtttat
ttaaaggCGg taataCGgtt atttatagaa ttaggggata aCGtaggaaa
gaatatgtga gtaaaaggtt ag – 3′
```

Non-methylated pUC19 DNA: Below is the expected sequence for the non-methylated pUC19 DNA after bisulfite conversion and PCR (sense strand). During treatment with sodium bisulfite, non-methylated cytosines are converted to uracil, which are later detected as thymine after PCR.

```
      5′ – ggttatagtt gttttttgtg tgaaattgtt attTGtttat
aattttatat aatataTGag tTGgaagtat aaagtgtaaa gtttggggtg
tttaatgagt gagttaattt atattaattg TGttgTGttt attgttTGtt
ttttagtTGg gaaatttgtT Gtgttagttg tattaatgaa tTGgttaaTG
TGTGgggaga ggTGgtttgT GtattgggTG tttttTGtt ttttTGttta
ttgattTGtt gTGttTGgtT GttTGgttgT GgTGagTGgt attagtttat
ttaaaggTGg taataTGgtt atttatagaa ttaggggata aTGtaggaaa
gaatatgtga gtaaaaggtt ag – 3′
```

Ordering Information

Product Description	Catalog No.	Size
Human Methylated & Non-methylated DNA Set	D5014	5 µg/20 µl
Human HCT116 DKO Non-Methylated DNA	D5014-1	5 µg/20 µl
Human HCT116 DKO Methylated DNA	D5014-2	5 µg/20 µl
Bisulfite-Converted Universal Methylated Human DNA Standard	D5015	1 µg/50 µl
Zymo*Taq*™ qPCR Premix	E2054 E2055	50 Rxns. 200 Rxns.
Zymo*Taq*™ Premix	E2003 E2004	50 Rxns 200 Rxns.
EZ DNA Methylation-Lightning™ Kit	D5030 D5031	50 Rxns 200 Rxns.
EZ DNA Methylation-Direct™ Kit	D5020 D5021	50 Rxns 200 Rxns.
EZ DNA Methylation™ Kit	D5001 D5002	50 Rxns 200 Rxns.
EZ DNA Methylation-Gold™ Kit	D5005 D5006	50 Rxns 200 Rxns.

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Integrity of kit components is guaranteed for up to one year from date of purchase.
Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

The Polymerase Chain Reaction (PCR) process is covered by U.S. Patent: #4,683,195; 4,683,202 assigned to Hoffmann-La Roche. Patents pending in other countries. No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of Zymo Research's products. Further information on purchasing licenses to practice the PCR process can be obtained from the director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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