ZYMO RESEARCH

Epigenetics
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Human WGA Methylated & Non-Methylated DNA Set

Standards for DNA methylation analysis workflows

Highlights

- Purified, whole genome amplified methylated and non-methylated human DNA for use as positive and negative controls in methylated detection assays.
- Utilize as mock samples to optimize methylation analysis workflows.
- Provided primer pair targeting the Ras association domain family member 1 gene allows for convenient assay quality control.

Catalog Numbers:
D5013, D5013-1, D5013-2

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

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Table of Contents

Product Contents

Human WGA Methylated & Non-Methylated DNA Set	D5013	D5013-1	D5013-2	Storage Temp.
Human WGA Non-methylated DNA	5 µg/20 µl	5 µg/20 µl	-	-20ºC
Human WGA Methylated DNA	5 µg/20 µl	-	5 µg/20 µl	-20ºC
RASSF1 Primers	20 µl	-	-	-20ºC

Specifications

Human WGA Non-Methylated DNA

- **Source –** Whole genome amplified DNA from HCT116 DKO cells [DNMT1 (-/-) / DNMT3b (-/-)].
- **Concentration –** 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Human WGA Methylated DNA

- **Source –** Whole genome amplified DNA from HCT116 DKO cells that has been enzymatically methylated by M.SssI methyltransferase.
- **Concentration –** 250 ng/µl in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

RASSF1 Primers

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

  RASSF1 Forward Primer:

  5′ – GGTGGTTAYGGTTAGGGATTAGTTGT – 3′

  RASSF1 Reverse Primer:

  5′ – AACCCCACAATCCCTACACCCAAATTTCCATTA – 3′

Product Description

The **Human WGA Methylated & Non-methylated DNA Set** consists of two control DNAs (non-methylated and 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.

The **Human WGA Methylated & Non-methylated DNA Set** is generated using phi29 DNA polymerase based whole genome amplification techniques from HCT116 DKO[1] cell line derived genomic DNA (**Human HCT116 DKO Non-methylated DNA**). The **Human WGA Methylated DNA** is **Human WGA Non-methylated DNA** that has been enzymatically methylated at all double-stranded CG dinucleotides using M.SssI methyltransferase[2] (EC 2.1.1.37; Figure 2) and can be used as a positive control for DNA methylation analysis.

Methylated cytosines comprising CG dinucleotides within DNA remain unconverted following bisulfite treatment, whereas non-methylated cytosines are converted to uracil and detected as thymine following PCR. The **RASSF1 control primers** amplify methylated, non-methylated, and mixed methylation copies of the Ras association (RalGDS/AF-6) domain family member 1 (RASSF1) gene and are intended for use after bisulfite conversion of the control DNA. Recovered DNA is ideal for many applications including downstream analyses such as PCR, restriction endonuclease digestion, sequencing, etc.

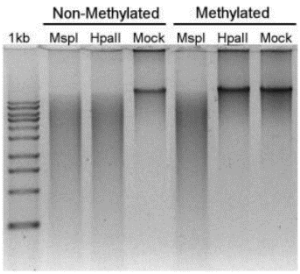

Figure 1. An assay for complete methylation by M.SssI methyltransferase. Digestion of non-methylated and methylated HCT116 DKO DNA with restriction enzymes MspI and HpaII. MspI digests both non-methylated and methylated DNA. HpaII is sensitive to CpG methylation.

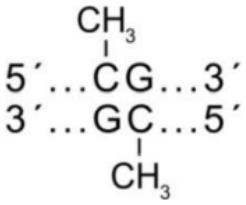

Figure 2. M.SssI methyltransferase methylates all cytosine residues in double stranded CpG context.

[1]Rhee et al. Nature. 416: 552-556 (2002).
[2]Nur et al. J. Bacteriol. 164: 19-24 (1985).

Recommended Usage

The **Human WGA Methylated & Non-methylated DNA Set** can be used in a variety of methylation analysis applications including bisulfite and methylation-specific PCR, 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. Vortex the standards for 10-15 seconds, briefly spin down for 5-10 seconds.
3. Repeat Step 2 twice, for three times total.
4. Proceed with quantification or usage.

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

Component	Volume	Final Concentration
RASSF1 Primers[2]	Variable	0.2 to 1.0 µM each
Bisulfite-converted DNA[3]	2 µl	Up to 20 ng/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. 59 °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

The PCR amplicon can now be used directly for sequencing analysis or cloning.

[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

RASSF1 Bisulfite PCR

The expected PCR amplicon for the **Human WGA Methylated & Non-methylated DNA** is 327 bp and corresponds to the region 4962 to 5288 nucleotides downstream from the start of the RASSF1 coding sequence on the reverse strand, including the regions (italicized) that hybridize to the primers (GenBank Accession # NG_023270).

Original sequence of the RASSF1 fragment for bisulfite treatment and PCR amplification (anti-sense strand 5' to 3'). The cytosines (underlined) in the CpG dinucleotide context (bold capital letters) are methylated enzymatically by M.SssI methyltransferase or not methylated in the non-methylated DNA.

```
      5′ - ggtggccaCG gccagggacc agctgcCGtg tggggttgca
CGCGgtgccc CGCGCGatgC GcagCGCGtt ggcaCGctcc agcCGggtgC
Ggcccttccc agCGCGccca gCGggtgcca gctccCGcag ctcaatgagc
tcaggctccc cCGacatggc cCGgttgggc cCGtgcttCG ctggctttgg
gCGctagcaa gCGCGggcCG ggCGgggcca cagggCGggc ccCGacttca
gCGcctcccc caggatccag actgggCGgC GggaaggagC tgaggagagc
CGCGcaatgg aaacctgggt gcagggactg tggggcc – 3′
```

Expected sequence of the above PCR amplicon following bisulfite treatment:

Human WGA Non-methylated DNA. Below is the expected sequence for the Human WGA Non-methylated DNA after bisulfite conversion and PCR. During treatment with sodium bisulfite, non-methylated cytosines are converted into uracil, which are detected as thymine after PCR.

```
       5′ - ggtggttaTG gttagggatt agttgtTGtg tggggttgta
TGTGgtgttt TGTGTGatgT GtagTGTGtt ggtaTGtttt agtTGggtgT
Ggttttttt agTGTGttta gTGggtgtta gttttTGtag tttaatgagt
ttaggttttt tTGatatggt tTGgttgggt tTGtgtttTG ttggttttgg
gTGttagtaa gTGTGggtTG ggTGgggtta tagggTGggt ttTGatttta
gTGttttttt taggatttag attgggTGgT GggaaggagT tgaggagagt
TGTGtaatgg aaatttgggt gtagggattg tggggtt – 3′
```

Human WGA Methylated DNA. Below is the expected sequence for the Human WGA Methylated DNA after bisulfite conversion and PCR. 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′ - ggtggttaCG gttagggatt agttgtCGtg tggggttgta
CGCGgtgttt CGCGcgatgC GtagCGCGtt ggtaCGtttt agtCGggtgC
Ggttttttt agCGCGttta gCGggtgtta gttttCGtag tttaatgagt
ttaggttttt tCGatatggt tCGgttgggt tCGtgtttCG ttggttttgg
gCGttagtaa gCGCGggtCG ggCGgggtta tagggCGggt ttCGatttta
gCGttttttt taggatttag attgggCGgC GggaaggagT tgaggagagt
CGCGtaatgg aaatttgggt gtagggattg tggggtt – 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.

Notes

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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 DKO technology is licensed from The Johns Hopkins University.

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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