CpGenome™ Direct Prep-96 Bisulfite Modification Kit

Catalog No. 17-10454

192 reactions

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Not for use in diagnostic procedures.

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Introduction

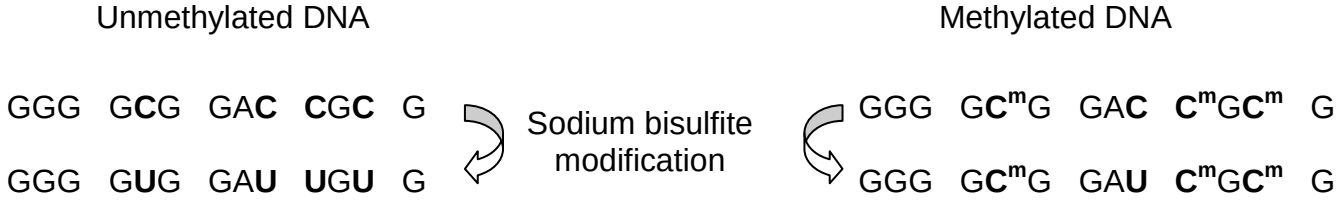
Methylation of cytosines located 5' to guanosine is known to have a profound effect on the expression of many eukaryotic genes. In normal cells, methylation occurs predominantly in CG-poor regions, while CG-rich areas_ called CpG-islands_ remain unmethylated. The exceptions are the extensive methylation of CpG islands associated with transcriptional inactivation of regulatory regions of imprinted genes and genes on the inactive X-chromosome. Aberrant methylation of normally unmethylated CpG islands has been documented as a relatively frequent event in immortalized and transformed cells and has been associated with transcriptional inactivation of defined tumor suppressor genes in human cancers. Hundreds of CpG islands are now known to exhibit the characteristic of hypermethylation in tumors, resulting in identification of candidate genes that can be interrogated to determine extent of tumor specific transformation.

Several methods have been developed to determine the methylation status of cytosine. These include the use of antibodies or protein methyl binding domains, digestion with methylation sensitive, insensitive, or dependent restriction enzymes as in restriction landmark genomic scanning, oligonucleotide array hybridization, bisulfite genomic DNA sequencing and Methylation Specific PCR (MSP). Some techniques are more useful for discovery (e.g. RLGS) while others are better used for evaluating sequence specific methylation of known methylated cytosines (e.g. MSP).

Genomic DNA sequencing, although time consuming and labor intensive, offers a more universal detection method. MSP is an established technology for the monitoring of abnormal gene methylation in selected gene sequences. Utilizing small amounts of DNA, this procedure offers sensitive and specific detection of 5-methylcytosine in promoters. It is being exploited to define tumor suppressor gene function, and to provide a new strategy for early tumor detection by interrogating DNA derived from tissues and bodily fluids.

The initial step of both bisulfite genomic sequencing and MSP is to perform a bisulfite modification of the DNA sample. As shown in Figure 1, in the bisulfite reaction, all unmethylated cytosines are deaminated and sulfonated, converting them to uracils, while 5-methylcytosines remain unaltered. Thus, the sequence of the treated DNA will differ depending on whether the DNA is originally methylated or unmethylated. Also, the initially complementary DNA strands will no longer be complementary after cytosine conversion. Primers for use in MSP can be designed to specifically amplify either a bisulfite-sensitive, unmethylated strand or a bisulfite-resistant, methylated strand, based upon these chemically-induced differences. For additional details on MSP please refer to the appendix.

Figure 1. DNA treatment with bisulfite reagent.



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CpGenome Direct Prep-96 Bisulfite Modification Kit Overview

Unlike other bisulfite modifications approaches that start with isolated genomic DNA, the CpGenome Direct Prep-96 Bisulfite Modification Kit allows simple and reliable bisulfite conversion of DNA directly from a variety of starting materials, including cultured cells, blood, fresh tissue and fixed tissue samples. This kit combines high sensitivity with ease-of-use to allow detection of bisulfite-converted DNA from as few as 10 cells or as low as 50 pg of input DNA. This streamlined protocol permits one-step conversion of non-methylated cytosines in DNA samples into uracil. In-column desulfonation facilitates recovery of DNA without cumbersome precipitation steps providing more consistent results. After PCR amplification, downstream analysis of the recovered DNA can be performed by restriction digestion, sequencing, microarray hybridization, etc.

To complement the CpGenome Direct Prep-96 Bisulfite Modification Kit, we offer a range of CpG WIZ® Amplification Kits containing the materials required for sensitive detection of bisulfite modified DNA using MSP. To see the collection of targets offered as well as additional kits, assays, antibodies, and proteins for the study of DNA methylation visit us at www.millipore.com/epigenetics.

Warnings and Precautions

This protocol requires the use of strong acids and bases to prepare the bisulfite modification solution. Avoid inhalation, ingestion or contact with skin. Use of a chemical fume hood or particle mask is recommended when weighing the bisulfite reagents.

Binding Buffer contains an irritant to skin and mucous membranes. The use of Personal Protective Equipment and gloves when handling bisulfite modification reagents as well as the DNA purification reagents is strongly recommended.

Storage and Stability

Store all components of this kit at room temperature (15℃ - 30℃) except the Proteinase K and Proteinase K Storage Buffer, which should be stored at -20℃. Performance is guaranteed for 6 months from date of receipt when all reagents are stored properly.

Materials Provided

CpGenome Direct Prep-96 Bisulfite Modification Kit 17-10454-1 Store at Room Temperature (15℃ to 30℃)		
Component	**Item No.**	**Volume**
2X Extraction Buffer	CS214115	15 mL
Modification Reagent	CS214695	2 bottles
Conversion Buffer	CS214117	7 mL
Resuspension Buffer	CS214118	18 mL
Equilibration Buffer	CS214111	4 mL
Binding Buffer	CS214120	125 mL
Wash Buffer I	CS214696	2 x 36 mL
Wash Buffer II	CS214122	40 mL
Elution Buffer	CS216117	8 mL
Desalting Plates	CS214697	2 plates
Collection Plates	CS214699	2 plates
Elution Plates	CS214689	2 plates
Conversion Plates/Cover films	CS214698	2 x 2 plates
17-10454-2 Store at -20℃		
Proteinase K	CS214126	20 mg
Proteinase K Storage Buffer	CS214124	1.2 mL

Materials Required But Not Supplied

Reagents

- Nuclease-free water
- 96-100% Molecular Biology Grade Ethanol
- Xylene (For FFPE Samples)

Equipment

- Thermal cycler, oven, or water bath
- Vortex or Eppendorf Thermomixer®
- Centrifuge able to fit deep well 96 plates and desalting/collection plate assembly (height of 60 cm or 2.36 in and speed >3,500 x g (e.g. Thermo Scientific Sorvall™ ST 40R)
- Multi-channel pipettes and pipette tips (aerosol barrier tips are suggested to minimize potential cross contamination).
- Analytical scale or balance
- Untreated glass slides and slide washing chambers (for FFPE Samples)

CpGenome Direct Prep-96 Bisulfite Conversion Protocol

Please read entire protocol before starting

I. Starting Material

A. Cells: This kit can be used to convert genomic DNA from cultured primary or immortalized cells. Although bisulfite conversion is achievable with as few as 10 cells, a range of 1,000 to 100,000 cells is recommended for optimal results

B. Tissues, Whole Blood, FFPE samples: This kit can be used for fresh or frozen tissues, including whole blood, buffy coat, FFPE samples and biopsies. Up to 2.5 µL of whole blood or up to 0.5 mg of fresh or frozen tissue is recommended for use with this kit.

C. Purified DNA: Previously isolated DNA can be used with this kit. Purified DNA samples containing 200 ng to 500 ng will provide optimal results, although it is possible to obtain conversion of as little as 50 pg of DNA.

II. Preparation of reagents

A. Resuspend Proteinase K

Add 1040 µL of Proteinase K Storage Buffer to the tube labeled Proteinase K. Dissolve completely and store at -20℃.

B. Reconstitute Modification Reagent

Add 7.9 mL of Resuspension Buffer and 3.0 mL of Conversion Buffer to a bottle of Modification Reagent. Mix thoroughly by shaking or vortexing for 15 minutes at room temperature. Add 1.6 mL of Equilibration Buffer and mix for 4 minutes. The Modification reagent is a saturated solution and it is normal to find trace amounts of undissolved reagent.

Note: This reagent is light-sensitive, so minimize exposure to light for best results.

Storage of Reconstituted Modification Reagent: This reagent should be used immediately following preparation for optimal results. If only performing bisulfite conversion for a partial 96-well plate, left-over reconstituted Modification Reagent can be stored for later use. For best results minimize exposure to light. Reconstituted Modification Reagent is stable overnight at room temperature, one week at 4℃ or one month at -20℃. If stored below room temperature, warm to 37℃ and vortex to mix thoroughly prior to use.

C. Wash Buffer I

Add 144 mL of 100% Ethanol to 36 mL of Wash Buffer I before use. Check on the bottle label to indicate ethanol has been added.

III. CpGenome Direct Prep-96 Sample Digestion and Modification Protocol

A. Sample digestion with Proteinase K

Protocols for cultured cells, blood, tissues and FFPE samples are below. Please see appendix for additional recommendations on working with larger amounts of input sample, and other sample types.

1. Protocol for cultured cells, blood, fresh or frozen tissue

    a) For up to 1 x 10^5 cells, 0.1 mg tissue or 0.5 µL blood (total volume of input sample should not exceed 12 µL), add 13 µL of 2X Extraction Buffer, 1 µL of Proteinase K and water to make up the total volume to 26 µL.

    b) Incubate the samples for 20 minutes at 50℃.

    c) Optional: Spin down the samples at 10,000 x g for 5 minutes if any debris is present. Use 20 µL of the supernatant for bisulfite conversion.

2. Protocol for FFPE samples

    d) Slice the FFPE block into 10 µM sections and place on non-treated slides.

    e) Wash the slides three times in Xylene for 5, 5 and 3 minutes, each with new Xylene solution.

    f) Wash the slides twice in 100% Ethanol for 1 minute each.

    g) Wash the slides twice in 70% Ethanol for 1 minute each.

    h) Wash the slides twice in 30% Ethanol for 1 minute each.

    i) Wash the slides twice in Water for 1 minute each.

    j) Scrape the tissue into an appropriate micro-centrifuge tube.

    k) Add 25 µL of 2X Extraction Buffer, 2 µL of Proteinase K and 23 µL of water to each tube and mix.

    l) Incubate the samples for 24 hours at 50℃.

    m) Optional: Spin down the samples at 10,000 x g for 5 minutes if any debris is present. Use 20 µL of the supernatant for bisulfite conversion.

B. Bisulfite conversion of DNA

1. Add 20 µL of the sample from step c (Section III.A.1) or step j (Section III.A.2) above to 130 µL of reconstituted Modification Reagent in a Conversion Plate. Mix the sample and centrifuge briefly to ensure all the liquid settles at the bottom of the plate.
2. Seal the Conversion Plate with provided cover film. Place the Conversion Plate in a thermal cycler and incubate the sample for 98℃ for 8 minutes, followed by 64℃ for 3.5 hours and 4℃ for up to 20 hours.
3. Pre-warm Wash Buffer II and Elution Buffer in a 42℃ oven or water bath.
4. Place a Desalting Plate into a Collection Plate and add 600 µL of Binding Buffer.

5. Load the sample from Step 2 into each well of the Desalting Plate containing the Binding Buffer. Mix several times by pipetting.
6. Centrifuge at full speed (>3,500 x g) for 5 minutes. Discard the flow-through and reuse the collection plate for subsequent washes.
7. Add 400 µL of Wash Buffer I to the each well and centrifuge at full speed (>3,500 x g) for 5 minutes. Discard the flow-through and reuse the collection plate for subsequent washes.
8. Add 200 µL of pre-heated Wash Buffer II to each well and incubate at room temperature for 15 to 20 minutes. Following the incubation, centrifuge at full speed (>3,500 x g) for 5 minutes. Discard the flow-through and reuse the collection plate for subsequent washes.
9. Add 400 µL of Wash Buffer I to each well and centrifuge at full speed (>3,500 x g) for 5 minutes. Repeat the wash once more, by discarding the flow-through and reusing the collection plate after each spin.
10. Place the Desalting Plate on top of an Elution Plate. Add 15 µL pre-heated Elution Buffer directly to the membrane of each well of the plate. Incubate at RT for 5 minutes. Centrifuge at full speed (>3,500 x g) for 3-5 minutes to elute the bisulfite-converted DNA. Larger elution volumes or sequential elution can be used if required, but this will result in lower DNA concentrations.
11. The eluted DNA is ready for downstream applications. Alternatively the DNA can be stored at -20℃ for up to 2 weeks if not used immed iately. For longer-term storage -80℃ is recommended.
12. Note: Bisulfite converted DNA is chemically less stable than non-converted, double stranded intact genomic DNA. Long term storage of bisulfite converted samples is not recommended.

C. Analysis of bisulfite-converted DNA

Bisulfite-modified DNA can be analyzed by several molecular methods including MSP (methylation specific PCR) using endpoint-PCR or quantitative RT-PCR, cloning and dideoxy-sequencing, microarray analysis or deep sequencing methods.

Useful information on primer design and other considerations for Methylation-Specific PCR (MSP) can be found in the Appendix. Should additional questions arise, assistance is available from our highly trained Technical Service team; please see front cover for contact information.

Product Performance

The chemistry used in the CpGenome Direct Prep-96 Bisulfite Modification Kit was evaluated using various starting materials and the CpG WIZ® BRCA1 Amplification Kit (Cat. No. S7830), CpG WIZ® Oct4 Amplification Kit (Cat. No. S7840) or the CpG WIZ® RASSF1A Amplification Kit (Cat. No. S7813) to detect modified DNA. Bisulfite conversion was confirmed in primary cultured cells and fresh tissue (Figure 2); whole blood (Figure 3); FFPE samples (Figure 4). Bisulfite conversion efficiency was typically greater than 99.5% as measured by conversion of CpGenome™ Cytosine DNA Standard (S8005U) spiked into the genomic samples. PCR amplification of bisulfite converted DNA was possible with low amount of starting material (Figure 5), with detectable amplicons detected when as few as 10 cells were used.

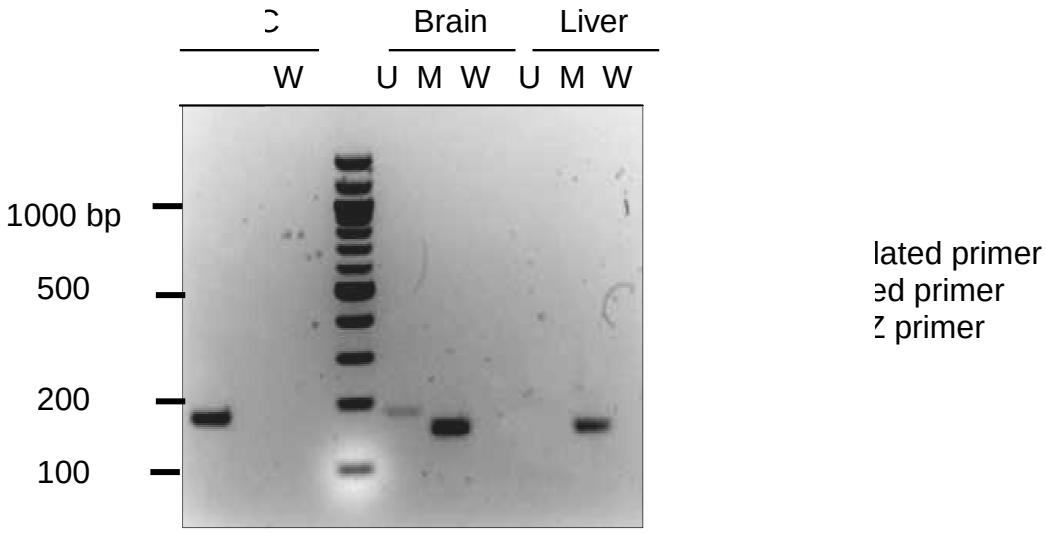

Figure 2. Mouse ES cell, mouse brain tissue and mouse liver tissue were treated with proteinase K and bisulfite converted as described in the protocol. CpG WIZ Oct4 Amplification kit was used to amplify the converted DNA using quantitative PCR. PCR products were analyzed on the 2% agarose gel.

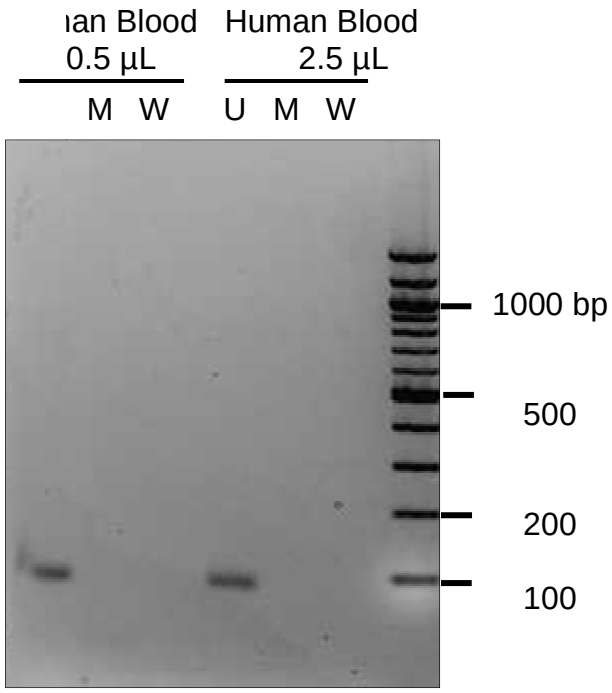

Figure 3. Human normal blood 0.5 µL or 2.5 µL was directly treated with proteinase K and bisulfite converted as described in the protocol. CpG WIZ BRCA1 Amplification kit was used to amplify the converted DNA using quantitative PCR. PCR products were analyzed on the 2% agarose gel.

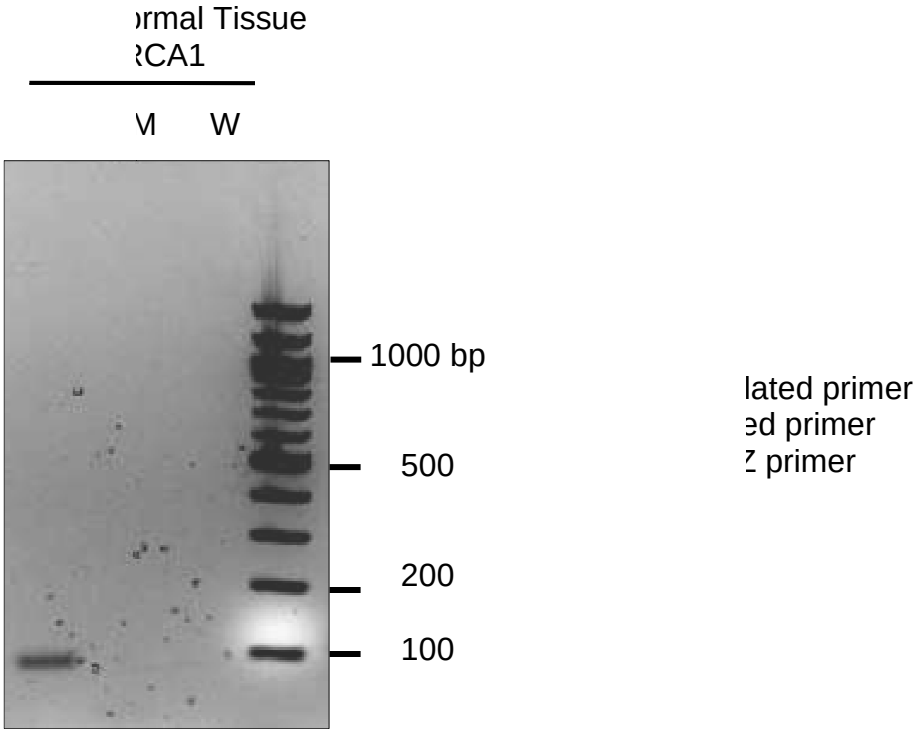

Figure 4. Human normal FFPE sample was treated with proteinase K and bisulfite converted as described in the protocol. CpG WIZ BRCA1 Amplification kit was used to amplify the converted DNA using quantitative PCR. PCR products were analyzed on the 2% agarose gel.

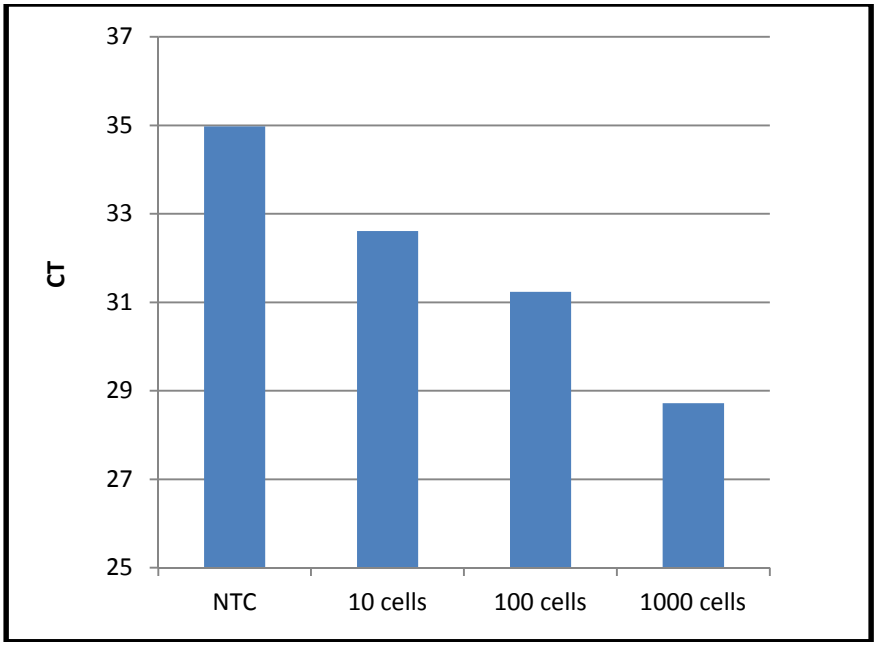

Figure 5. 10, 100 and 1000 HeLa cells were bisulfite converted as described in the protocol. Real-time quantitative PCR was used to analyze converted DNA by BRCA1 U primer. NTC: No Template Control, CT: Cycle Threshold.

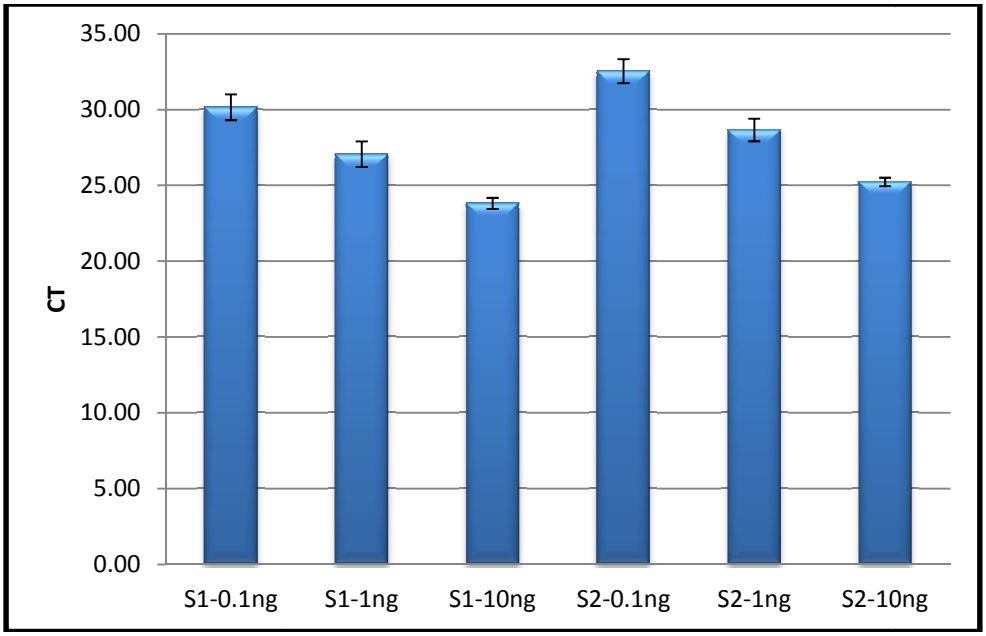

Figure 6a. Analysis of three triplicates of breast tissue sample 1 (S1- 0.1 ng, S1– 1 ng, S1– 10 ng) and breast tissue sample 2 (S2- 0.1 ng, S2- 1 ng, S2- 10 ng). Samples were prepared as described in section B of the protocol. Bisulfite converted DNAs were subjected to MSP using CpG WIZ Unmethylated RASSF1A (catalog number S7813). Coefficient of variance of three random triplicates on the 96-well plate for assay with breast gDNA samples are < 10%.

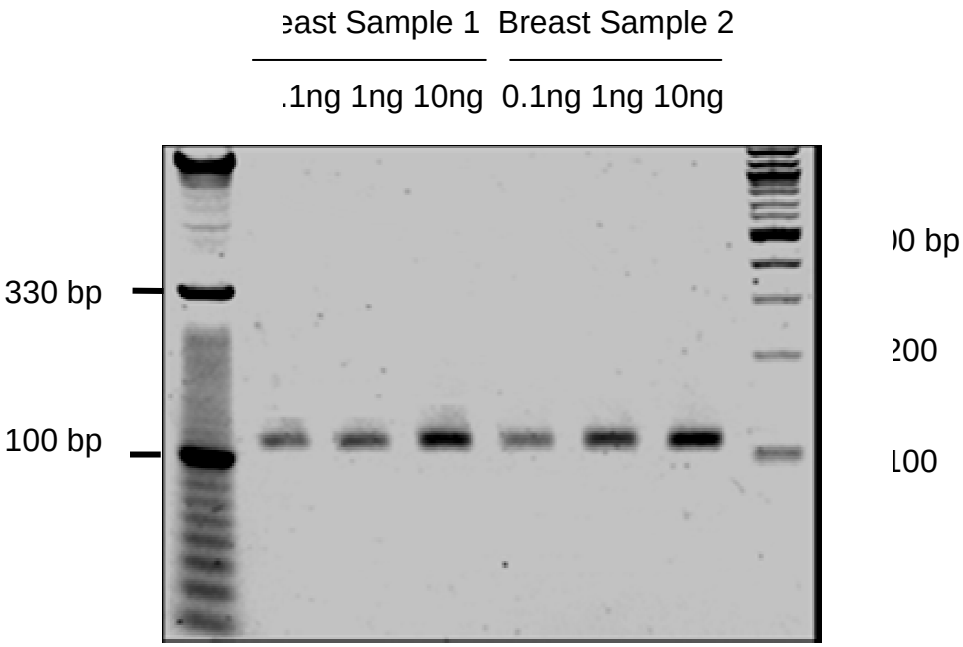

Figure 6b. Analysis of three triplicates of breast tissue sample 1 (S1- 0.1 ng, S1- 1 ng, S1- 10 ng) and breast tissue sample 2 (S2- 0.1 ng, S2- 1 ng, S2- 10 ng). Samples were randomly arrayed in a 96-well conversion plate and bisulfite converted as described in section B of the protocol. Bisulfite converted DNAs were subjected to MSP using CpG WIZ Unmethylated RASSF1A (S7813) and analyzed on a 4% agarose gel.

Troubleshooting

Step	Problem	Experimental Suggestions
Sample Digestion with Proteinase K	Incomplete Digestion	• Amount of proteinase K and incubation time can be empirically adjusted for some “difficult to digest” samples. •“Difficult to digest” samples, including connective tissue (e.g. cartilage), adipose tissue, some fixed tissues, etc. usually form visible debris after digestion. Carefully remove debris by centrifugation before proceeding to bisulfite conversion of the DNA.
Bisulfite Conversion of DNA	Low Conversion Efficiency/Low yield	• Use amount of cells, tissues or FFPE samples recommended in protocol for optimal conversion efficiency. • Confirm starting DNA material was intact using an unconverted template control W primer set. • Sodium bisulfite modifies only single-stranded DNA. Double stranded DNA must be denatured in 300mM NaOH prior to modification. Ensure NaOH solution is made fresh from solid pellets. • Ensure DNA modification buffer is at correct pH. Use pH paper check pH and adjust pH to 5.3-5.4 if required. • Use multi-channel pipette and aerosol pipette tips to dispense reagents into wells. Empty the Collection Plate after each spin to prevent flow-through contamination. • If following protocol in section III for 96-well plate of cultured cells, blood, fresh or frozen tissue samples, the modification reagent can be added directly into the samples by either piercing through or carefully removing the cover film. Completely re-seal the Conversion Plate with a new cover film before placing in a thermal cycler to prevent evaporation.
Bisulfite Converted DNA Analysis	Inaccurate Quantification	• The converted DNA is single stranded and AT rich, therefore absorption coefficient at 260nm should be used as 40µg/mL when determine concentration of the DNA.
MSP Primer Design	Sub-optimal PCR primers	• PCR primer design (24-32 bases) with unmethylated cytosines should be treated as thymines (Cs convert to Ts during bisulfite treatment). Avoid mixed bases C and T at 3’ end and no more than 3 mixed bases in the primer. • Ensure primer design is validated in end point PCR without primer dimer formation. Check primer quality by melt curve analysis in a real time capable thermal cycler.

Step	Problem	Experimental Suggestions
PCR Amplification	Sub-optimal PCR conditions	• To prevent contamination of PCR amplicons with amplified products, use fresh aliquots of PCR reagents (i.e. dNTPs, buffer, etc). Use separate set of pipettes for each liquid dispensing. Devote a work area to pre- and post-amplification procedures. Always use aerosol-resistant pipette tips. Always use a clean lab coat and gloves. • To minimize potential present of inhibitor following bisulfite conversion and DNA purification, dilute bisulfite converted DNA in water and use less DNA as template in PCR reactions. • Optimize PCR conditions: Typical conditions for successful PCR amplification of converted DNA: 35-40cycles, amplicon size: 150-300 bp (up to 1kb can be made with optimized bisulfite treatment and PCR conditions), and annealing temperature: 55-60ºC (or lower depend on templates GC content).

Appendix: Additional Considerations for CpGenome Direct Prep-96 Bisulfite Modification Kit

Guidelines for Larger Amount of Input Sample and Other Sample Types

- **Cultured Cells and Liquid-containing Cells:** Monolayer cells and cells in suspension, body fluids or those derived from FACS or buffy coat may be directly processed after collection. Trace or tiny amounts of culture medium do not adversely affect the procedure; however, cells are preferably resuspended in PBS or Tris-Buffer solutions prior to Proteinase K digestion. If the composition of the cell-liquid is unknown, centrifuge to pellet the cells, remove the supernatant, and resuspend cells in PBS or Tris-Buffer solutions. Although bisulfite conversion is achievable with as few as 10 cells, a range of 1,000 to 100,000 cells is recommended for optimal results**.**
- **Whole Blood:** 0.5 µL whole blood is used as described in section III-A1. When larger volumes, for example 5-fold or 2.5 µL whole blood is used, adjust Proteinase K volume to 5 µL, 2X Extraction Buffer to 60-65 µL, and add water to make up the volume to total of 130 µL.
- **Fresh or Frozen Solid Tissues:** 0.1 mg of fresh or frozen tissue is used as described in section III-A1. When larger weight, for example 5-fold or 0.5 mg fresh or frozen tissue is used, adjust Proteinase K volume to 5 µL, 2X Extraction Buffer to 60-65 µL, and add water to make up the volume to total of 130 µL.
- **FFPE (Formalin-Fixed Paraffin-Embedded) and Other "Fixed" Tissues:** Paraffin embedded tissues must be deparaffinized using Xylene and wash with Ethanol prior to Proteinase K digestion. The extent of the digestion time should be adjusted empirically to achieve fully digested samples.
- **LCM (Laser Capture Micro-Dissection):** Tissue samples from LCM should be in PBS or Tris-Buffer solutions prior to Proteinase K digestion. The extent of the digestion time should be adjusted empirically to achieve fully digested samples**.**

Methylation Specific PCR (MSP) Overview

For this amplification based analysis method, PCR primers are designed to specifically amplify the promoter regions of the gene of interest. If the sample DNA was originally unmethylated, an MSP reaction product will be detectable when using the primer set (labeled as 'U') designed to be complementary to the unmethylated bisulfite converted DNA sequence. No product will be generated using a primer set (labeled as 'M') designed to be complementary to the derivative methylated DNA sequence. Conversely, an MSP product will be generated only using the M primer set if the sample was originally methylated, and the U primers will not amplify such a template.

Methylation specific PCR permits sensitive detection of altered DNA. Because it is a PCR-based assay, it is extremely sensitive, facilitating the detection of low numbers of methylated alleles and the evaluation of DNA from small samples, including paraffin-embedded, formalin fixed materials. MSP, when coupled with bisulfite sequencing, also allows examination of all CpG sites, not just those within sequences recognized by methylation sensitive restriction enzymes. Increasing the number of such sites that can be assessed allows rapid, fine mapping of methylation patterns throughout CpG regions. In addition, the bisulfite modification is ideally suited for analysis of CpG islands since it converts the majority of unmethylated cytosines to uracils, making a region of the genome which is CG rich more easily amplified by PCR.

MSP Primer Design

Methylation Specific PCR consists of chemical modification followed by amplification. Chemical modification creates the sequence differences between the methylated and unmethylated DNA. Ideally, three sets of primers should be designed to anneal to the DNA, based upon these sequence differences. The three primer sets each are designed to detect the different states of a converted DNA sequence.

- Primer set (U) will anneal to unmethylated DNA that has undergone a chemical modification.
- Primer set (M) will anneal to methylated DNA that has undergone a chemical modification.
- Primer set (W) will anneal to any DNA (unmethylated or methylated) that has NOT undergone chemical modification, hence, the "wild type", or W. This serves as a control for the efficiency of chemical modification.

The standard rules for primer design apply toward the creation of MSP primers. The primers should be approximately 20-21 bp in length and should possess similar dissociation temperatures. The amplified product produced with each set of primers should be 100-200 bp in size. Internal secondary structure should be avoided. In order to minimize primer dimer formation, primers should not be complementary, especially at the 3' end. Discrimination between methylated and unmethylated sequences by MSP seems to be greatest when the 3' ends (or anchor region) of the primers are most different from one another.

Alternatively, MethPrimer software package, a functional program that selects oligonucleotide primers from a sequence file for methylation-specific PCR (MSP) analysis can be found at http://www.urogene.org/methprimer/index1.html. This interactive online program will select and generate primer sets that may potentially be used for MSP analysis.

MSP Experimental Design

To perform a thorough analysis of each modified DNA sample, experiments should include amplification reactions using the U, M and W primer sets as described above. Unmodified DNA should be amplified with the W primer set. This reaction serves as a positive control for PCR and verification of the integrity of the genomic DNA sample. In addition, a negative PCR control (i.e. no

DNA) should be performed for each set of primers. Depending on the DNA sample, a product may be produced using either the U or M primer sets or both sets may produce a product. Amplification using the W primer set with a modified DNA sample should not produce a product unless the modification was incomplete. However, PCR with the W primer set on unmodified DNA serves as a general positive PCR control product. Appearance of a product in the negative PCR control would indicate possible contamination.

Use of "Hot Start" PCR in MSP

The three sets of primers used in MSP are derived from sequences closely related to each other, which introduces an opportunity for mispriming. In order to avoid this and other PCR-related artifacts, "hot start" PCR is recommended. "Hot start" PCR permits the Taq polymerase to begin the reaction only after the template and primers are in single stranded form. There are several modifications of the standard PCR protocol which allow a "hot start" to occur. There are multiple approaches to performing a "hot start" PCR reaction. Simple approaches that heat a reaction mix to 95℃ in a thermal cycler prior to the addition of Taq can be used or more sophisticated approaches using either Taq-specific antibodies or polymerases modified such they are inactive until brought to 95℃ can also be used.

Note: When performing MSP, do not use a polymerase capable of 3-5' mismatch repair (i.e. thermal stable polymerase with proof reading activity). Also avoid use of uracil N-glycosylase containing PCR master mixes when amplifying bisulfite converted DNAs.

Laboratory Setup and Precautions

One of the most important considerations when using the CpGenome Turbo Bisulfite Kit, and when performing PCR, is the environment where the initial reaction mixtures are set up. The ideal environment is free of amplified DNA products, which can cause false-positive results. Some sources of PCR product contamination are pipettes, tips, gel box, buffer, tube racks, notebooks, lab coats and any other items exposed to amplified PCR products. The following precautions should be followed in all steps of the assay protocol.

1. Always wear gloves.
2. Use sterile water for all solutions, aliquot the solutions in small amounts, and use fresh aliquots as working solutions. Discard working solutions after use.
3. Keep the assay solutions (10X PCR buffers, dNTPs, polymerase, etc.) separate from the amplified DNA.
4. Always use aerosol resistant pipette tips.
5. Separate micropipettors and work areas are recommended for the following three steps of the assay:
    - DNA modification and purification
    - Amplification set-up
    - Post-amplification analysis

References

1) Mook, J. *et al.* (2013). *Nature Methods*. (10) [Epub ahead of print]
2) Zon, G., *et al*. (2009). *Analytical Biochemistry*. 392(2): 117-125.
3) Dietrich, D., *et al.* (2009). *J Histochem Cytochem*. 57(5): 477-489

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