HypoMethylCollector™

(version C1)

Catalog No. 55004

Active Motif North America
1914 Palomar Oaks Way, Suite 150
Carlsbad, California 92008, USA
Toll free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351

Active Motif Europe
Office Park Nysdam
Avenue Reine Astrid, 92
B-1310 La Hulpe, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050

Active Motif Japan
Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733

TABLE OF CONTENTS **Page**

Overview

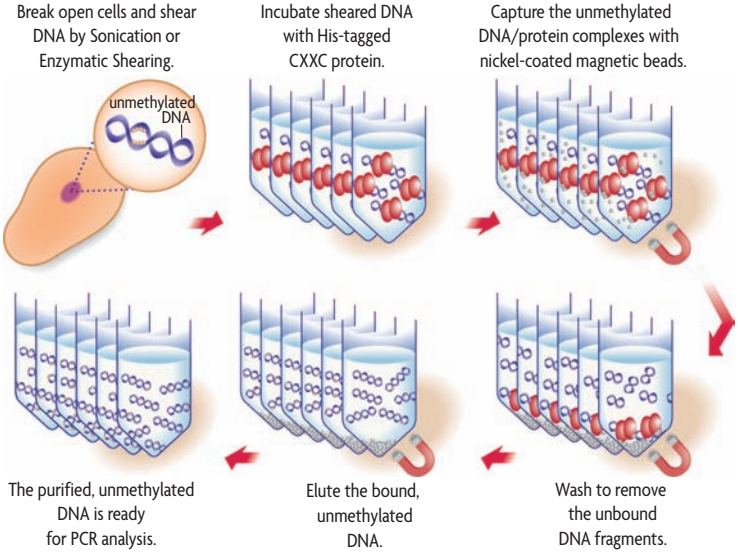
Active Motif's HypoMethylCollector™ Kit provides an efficient method for isolating non-methylated CpG dinucleotides from limited amounts of cell or tissue DNA. Most CpG island enrichment methods require clusters of methylated CpGs. Since CpG islands are normally umethylated[1, 2], isolation and evaluation of many human promoters contained within CpG islands has been difficult. Active Motif's HypoMethylCollector Kit resolves this issue by utilizing a His-tagged recombinant CXXC protein domain from mouse Mbd1 in order to specifically bind non-methylated CpGs. This novel technology allows for positive identification of hypomethylated promoters or can be used to confirm the negative results obtained by methyl-CpG capture methods, such as Active Motif's MethylCollector™ Ultra Kit (Cat. No. 55005). This simple and sensitive technique is suitable for use in many downstream applications, such as real time or endpoint PCR analysis of the methylation status of particular promoters in normal and diseased samples, rapid screening of the methylation status of multiple loci, sequencing, or amplification and labeling for microarray analysis. It can also be used to detect changes in DNA methylation in other situations, including normal cellular differentiation and aging.

In the HypoMethylCollector method*, His-tagged recombinant CXXC protein specifically binds unmethylated DNA fragments that have been prepared by enzymatic digestion or sonication. The His-CXXC is added to the DNA fragments, and these protein-DNA complexes are captured with nickel-coated magnetic beads. Subsequent wash steps are performed using an optimized buffer to separate the unmethylated fragments from the rest of the genomic DNA. The hypomethylated DNA is then eluted from the beads in the presence of high salt. Due to the high efficiency of HypoMethylCollector and the enormous amplification capability and specificity of PCR, analysis of the methylation status of a specific genomic DNA locus can be performed on DNA isolated from less than 1600 cells (~10 ng DNA).

product	format	catalog no.
HypoMethylCollector™	30 rxns**	55004

*Technology covered under U.S. Patent No. 8,105,787

**HypoMethylCollector provides sufficient reagents to perform 30 reactions with excess reagents for 5 control reactions.



Flow chart of the HypoMethylCollector process.

In HypoMethylCollector, genomic DNA of interest is sheared by either enzymatic digestion or sonication. The sheared DNA is then incubated with His-tagged recombinant CXXC protein, which has a high affinity for unmethylated DNA. These protein-DNA complexes are captured with nickel-coated magnetic beads and washes are then performed to remove the rest of the genomic DNA. The unmethylated DNA is then eluted from the beads and PCR is performed on the resulting supernatant, using specific primers to amplify the locus of interest.

Introduction

Over the last decade, the study of DNA methylation and its role in epigenetic cell signaling has grown rapidly[3-6]. Cellular methylation of CpG-dinucleotides, which occurs at the fifth position of the cytosine pyrimidine ring, is of particular interest.

Although CpG dinucleotides are generally methylated throughout the genome of normal somatic cells, CpG islands (clusters of CpG dinucleotides in gene regulatory regions) are usually hypomethylated[7]. Aberrant hypermethylation of CpG islands and subsequent transcriptional repression is one of the earliest and most common somatic genome alterations in multiple human cancers[8, 9]. Somewhat paradoxically, a decrease in the total amount of cytosine methylation is observed in many neoplastic tissues, but the genome context of this hypomethylation has not been identified[10]. Aberrant methylation of CpG islands thus seems to be a tumor type-specific event[9, 11] and current efforts have concentrated on finding ways to exploit the diagnostic and therapeutic implications of these abnormalities[12, 13].

Methyl-CpG binding proteins appear to be central players in the process of DNA methylation-dependent gene silencing[14]. This family of proteins takes its definition from the methyl-CpG binding domain (MBD), the minimum portion with specific affinity for a single, symmetrically methylated CpG pair. The MBD was characterized by deletion studies of MeCP2[15]. After the identification of the MBD, four additional genes were found to contain this domain, namely MBD1, MBD2, MBD3 and MBD4[16]. In general, all MBD proteins, except MBD4, have been reported to be associated with histone deacetylase subunits as part of large multi-subunit complexes[17, 18]. A few studies support the notion of selectivity in the association of a particular MBD with specific promoters[19, 20], but other results indicate that the CpG distribution along the sequence may influence the interaction of each MBD protein with DNA[21].

MBD1 is unique in that it contains three zinc-coordinating CXXC domains[22-24]. Other chromatin-associated proteins, such as DNA methyltransferase 1 (DNMT1), mixed lineage leukemia (MLL) and CpG binding protein (CGBP), also contain CXXC domains. Both MLL and CGBP have been shown to bind nonmethylated CpG sites *in vitro*[22, 25, 26]. The specificity of the CXXC domain is able to enrich for DNA fragments containing only a single non-methylated CpG. In addition, HypoMethylCollector can be used in parallel with the MethylCollector Ultra Kit (Catalog No. 55005) to provide a more thorough analysis of the methylation state at each locus of interest[27].

Traditional Methods to Study DNA Methylation

To date, there are several methods used for methylation analysis:

1. **Methylation-sensitive restriction enzyme analysis:** Isoschizomers of bacterial restriction endonucleases with different sensitivities for 5-methylcytosine can be used to determine the methylation status of specific CpG-dinucleotides[28]. Methylation-sensitive restriction enzymes have several limitations including that methylation-sensitive restriction merely informs on the methylation status of the cytosine residues which are recognized by the restriction enzymes used.
2. **Bisulfite conversion:** Bisulfite conversion[29] consists of the treatment of double-stranded genomic DNA with sodium bisulfite, leading to deamination of non-methylated cytosines into uracil. PCR is then performed with primers that differentiate between methylated and unmethylated sequences. Bisulfite-based techniques can be cumbersome, involving time- and labor-intensive chemical treatments that damage DNA and limit throughput. Additionally, PCR primer design becomes difficult due to reduction in genome complexity after bisulfite treatment, leading to an inability to elucidate the methylation pattern at CpG dinucleotides in a genomic locus of interest.
3. **Methylated DNA Immunoprecipitation (MeDIP):** In this assay, an antibody specific for methylated cytosines (anti-5-methylcytosine antibody) is used to immunoprecipitate methylated DNA from genomic DNA fragmented by enzymatic digestion or sonication[30]. The resulting enrichment is usually analyzed by PCR based methods; thus MeDIP can be combined with DNA microarrays for genome-wide analysis of CpG methylation. However, this technique is relatively time-consuming, requires a large amount of fragmented DNA starting material and only works with denatured DNA.

Kit Performance and Benefits

The HypoMethylCollector Kit is for research use only. Not for use in diagnostic procedures.

Range of detection: HypoMethylCollector can be performed on 10 ng - 1 µg of genomic DNA.

Nature of the HypoMethylCollector Assay: The HypoMethylCollector is designed as a simple, efficient technique to enrich for hypomethylated CpG islands. The methylation status of specific promoters contained within CpG islands can be analyzed using either endpoint or real time PCR analysis of the locus of interest with customer designed PCR primers. Control human, male genomic DNA that was digested with *Mse* I is included in the kit along with PCR primers specific for both unmethylated and methylated promoters.

GAPDH Glyceraldehyde-3-phosphate dehydrogenase is important for metabolism. Because this gene is often constitutively expressed, it is considered to be an actively transcribed housekeeping gene containing an unmethylated promoter in healthy tissues. The region amplified by this primer pair is 69 base pairs and contains 7 CpGs.

Xist X inactive specific transcript is a methylated promoter in human, male genomic DNA, but is unmethylated in females. The region amplified by this primer pair is 178 base pairs and contains 8 CpGs.

With either low or high salt binding conditions, the *Mse* I digested control human, male genomic DNA provided in the kit should have at least a 10-fold enrichment of unmethylated DNA bound and eluted from the protein complex as detected with the GAPDH PCR Primer Mix. This means that of the total DNA recovered, there is ten times as much hypomethylated DNA recovered in the eluted fraction as compared to the unbound fraction for the same locus.

There should be less than 5% of unmethylated DNA detected in eluted fractions using the Xist PCR Primer Mix for either low or high salt binding conditions.

The included control PCR primers are suitable for use in both endpoint and real time PCR analysis:

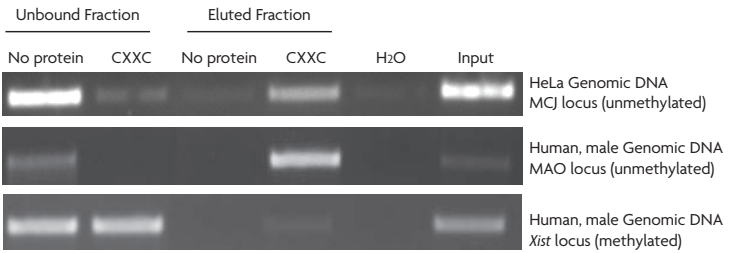


Figure 1: Specific isolation of unmethylated DNA using HypoMethylCollector Kit.

100 ng of *Mse* I digested genomic DNA was tested in the presence and absence of His-tagged CXXC protein in the HypoMethylCollector Kit. Both unbound and eluted fractions were collected and analyzed in PCR for 36 cycles. Both the MCJ locus, which is unmethylated in HeLa, and the MAO locus, which is unmethylated in males, were efficiently captured and eluted. The Xist locus, which is methylated in males, did not bind to the His-tagged CXXC protein and therefore can only be detected in the unbound fraction.

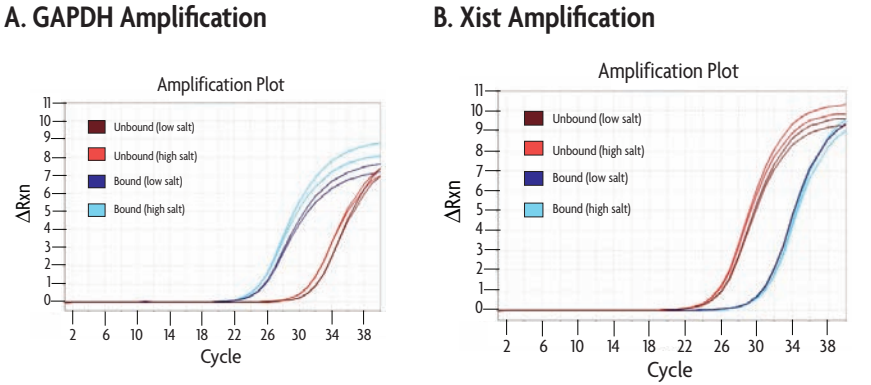


Figure 2: Real time PCR analysis of the control male genomic DNA with methylated and unmethylated promoters.

HypoMethylCollector was performed using 200 ng of *Mse* I digested human, male genomic DNA under both low and high salt conditions. Unbound and eluted DNA was purified and analyzed using real time PCR for both unmethylated and methylated promoters. **A)** Amplification plot using the provided GAPDH PCR primer mix with the unbound and eluted fractions. GAPDH is unmethylated in the control DNA and shows early amplification of the eluted fractions. **B)** Amplification plot using the provided Xist PCR primer mix with the unbound and eluted fractions. Xist is methylated in the control DNA and will not bind to the His-CXXC protein as represented with the late amplification in the eluted fractions and the early amplification in the unbound fractions.

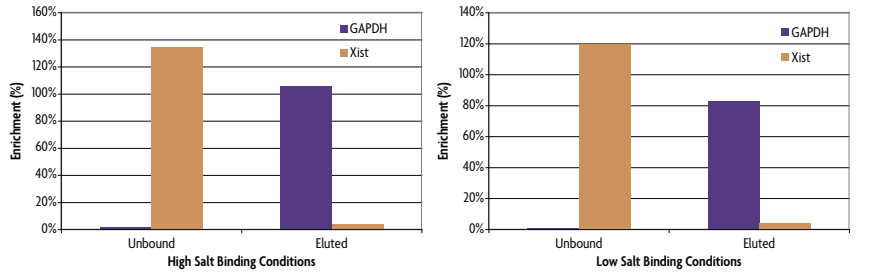


Figure 3: Fold enrichment of HypoMethylCollector binding reactions.

HypoMethylCollector was performed on 200 ng of *Mse* I digested human, male genomic DNA under both low and high salt conditions. Unbound and eluted DNA was purified and analyzed using real time PCR. The amount of DNA recovered in the eluted fraction was divided by the amount of input DNA used in the binding reaction to produce a percent enrichment. The unmethylated promoter, GAPDH, had greater than 10-fold enrichment of eluted DNA as compared to the unbound DNA for the same locus, while the methylated Xist promoter was found almost exclusively in the unbound fraction.

Kit Components and Storage

Kit components arrive on dry ice. Upon receipt, we recommend storing each component at the temperatures listed in the table below. The magnetic beads may be frozen; however, we recommend long-term storage at 4°C. **Do not subject the magnetic beads to repeated freeze/thaws.**

Reagents	Quantity	Storage / Stability
His-CXXC protein (0.65 µg/µl)	350 µl	-20°C for 6 months
Binding Buffer AM8 (High salt buffer)	50 ml	RT for 6 months
Binding Buffer AM9 (Low salt buffer)	5 ml	RT for 6 months
Elution Buffer AM3	5 ml	RT for 6 months
Protease Inhibitor Cocktail	100 µl	-20°C for 6 months
DTT (1 M)	100 µl	-20°C for 6 months
Human genomic DNA, *Mse* I digested	50 µl	-20°C for 6 months
GAPDH PCR Primer Mix (2.5 pmol/µl)	400 µl	-20°C for 6 months
Xist PCR Primer Mix (2.5 pmol/µl)	400 µl	-20°C for 6 months
10X PCR Buffer	1.5 ml	-20°C for 6 months
10X PCR Loading Dye	1.5 ml	-20°C for 6 months
Magnetic Nickel Beads	350 µl	4°C for 6 months
Glycogen	35 µl	-20°C for 6 months
Bar Magnet	1	Room temperature
Mini Glue Dots	2 Dots	Room temperature
8-strip PCR tubes and caps	12 strips	Room temperature

Additional Materials Required

- Fragmented DNA sample
- Sample PCR primer sets
- Sterile DNAase-free water
- Filter pipette tips
- Microcentrifuge tubes and microcentrifuge
- Magnetic stand. You can assemble a magnetic stand using the provided bar magnet and glue dots (see Appendix - Section C) or use commercially available stands
- Rotisserie shaker
- Phenol/chloroform

- 5 M Ammonium acetate (see Troubleshooting Guide, Appendix - Section D, for details regarding the use of 3 M sodium acetate, pH 5.2)
- 100% ethanol
- 70% ethanol
- *Taq* polymerase (5 U/µl) (Example: New England Biolabs M0267L or GeneSpin STS-T1000)
- dNTP mixture (5 mM each)
- SYBR Green mix for real time PCR analysis
- PCR cycler

NOTES BEFORE STARTING

Fragmentation of Genomic DNA

The provided His-CXXC protein binds specifically to non-methylated cytosines and the HypoMethylCollector method enriches for DNA fragments that contain one or more unmethylated CpG dinucleotides. To enable clear interpretation of results, genomic DNA should be prepared such that DNA fragments contain non-methylated CpGs in the region of interest (see Troubleshooting Guide, Appendix D for further discussion). DNA can be fragmented by restriction digest or by mechanical means (*e.g.*, sonication).

Restriction digestion is especially useful for analysis of individual CpG islands. The genomic DNA is digested with a methylation-insensitive restriction enzyme (or enzymes) so that only CpGs of interest are contained within a particular restriction fragment. This fragment should be long enough (75 bp or longer) to allow for PCR analysis. Some useful methylation-insensitive restriction enzymes are shown in the below table. As might be expected, the enzymes whose recognition sites contain G and C bases cut more frequently in CpG islands than enzymes whose sites are composed only of A and T bases.

	Recognition Sequence	Number of fragments (per kb) in CpG islands	Number of fragments (per kb) in non-CpG islands
***Mse* I**	TTAA	0.80	**2.88**
***Bfa* I**	CTAG	1.56	1.55
***Tas* I**	AATT	0.80	**2.88**
***Csp6* I**	GTAC	**2.23**	1.41

Mechanical fragmentation is ideal when a single DNA sample will be used for simultaneous analysis of many CpG islands (*e.g.*, when the isolated DNA will be analyzed by microarray methods) or when a CpG region of interest is not flanked by suitable restriction sites. In general, the DNA should be sheared to an average fragment size of less than 500 bp to minimize the number of CpG islands on each fragment.

Example fragmentation protocols are provided for both restriction digest and mechanical fragmentation on page 9. We suggest using 4 µg of purified genomic DNA when performing restriction digestion and 20 µg of purified genomic DNA for sonication. HypoMethylCollector can be performed on 10 ng – 1 µg of fragmented genomic DNA.

Example Fragmentation Protocols

Restriction digest

This protocol can be modified depending on the amount of isolated genomic DNA or the restriction enzyme being used. We recommend preparing high-quality genomic DNA using a commercially available kit or a standard established protocol. The quality of the genomic DNA can be assessed by agarose gel electrophoresis and DNA concentration can be determined by UV spectrophotometry.

a) Set up the following restriction digest (with *Mse* I as an example, New England Biolabs (NEB)):

Genomic DNA (400 ng/µl)	10 µl
10X NEB Buffer 2	10 µl
100X BSA	1 µl
Mse I (10 U/µl)	1 µl
dH_2O	78 µl
Total volume	**100 µl**

Note 1: The DNA volume may vary depending on its initial concentration.

Note 2: HypoMethylCollector has been used with as little as 10 ng of restriction-digested genomic DNA. As a reference, a human cell contains about 6 picograms DNA; 10 ng of genomic DNA corresponds to 1,600 cells.

b) Mix well by pipetting and incubate at 37°C for 2 hours to overnight.

c) Heat-inactivate *Mse* I by incubating the reaction mixture at 65°C for 20 minutes. If using an alternative restriction enzyme that cannot be heat-inactivated, the DNA can be purified by phenol/chloroform extraction and precipitation, or through use of a DNA purification column. See Troubleshooting Guide, Appendix D for comments about heat-inactivation.

Note 1: For greater accuracy, the digested DNA should be quantified.

Note 2: This digested DNA should be stored at -20°C until use.

Mechanical fragmentation (sonication)

Because *Mse* I or other restriction enzymes cannot always be used to fragment and isolate the DNA sequences of interest, sonication of the genomic DNA is an alternative method.

a) Pipette 20 µg genomic DNA into a 1.5 ml microcentrifuge tube and adjust final volume to 300 µl by addition of 10 mM Tris-HCl pH 8.5.

b) Sonicate on ice using 15 pulses of 20 seconds (30% amplitude if using Active Motif's EpiShear™ Sonicator, Catalog Nos. 53051 & 53052), with a 20-second pause on ice between each pulse. The sheared DNA can be visualized by ethidium staining after electrophoresis on a 3% agarose gel. The majority of the DNA fragments should be between 100 and 350 bp in length.

HypoMethylCollector Protocol

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Step 1: Planning the experiment

1. Determine the number of reactions needed. For PCR or sequencing analysis, one reaction per sample is usually sufficient. If downstream analysis will involve whole genome amplification and labeling for microarray analysis, please refer to our recommendations in Appendix A.
2. Two different binding buffers are included in the kit. For high salt conditions (high stringency reactions or DNA fragments containing more than 6 CpGs) we recommend using Binding Buffer AM8. For low salt conditions (lower stringency reactions or DNA fragments containing between 1-6 CpGs) we recommend using Binding Buffer AM9. It may be necessary to perform experiments with both binding conditions to determine the optimal conditions for the target of interest.

Step 2: Binding reaction

1. Thaw components from storage as needed for preparation. Keep frozen components on ice when not in use.
2. Prepare Complete Binding Buffer for the desired binding conditions.

   For high salt binding conditions (high stringency or DNA fragments containing more than six non-methylated CpGs): Prepare the appropriate amount of Complete Binding Buffer according to the table below. Store on ice.

Reagent	One rxn	8 rxns
Binding Buffer AM8	99.25 µl	794 µl
1 M DTT	0.25 µl	2 µl
Protease Inhibitor Cocktail	0.5 µl	4 µl
Total Volume	**100 µl**	**800 µl**

   For low salt binding conditions (lower stringency or DNA fragments containing between one and six non-methylated CpGs): Prepare the appropriate amount of Complete Binding Buffer according to the table below. Store on ice.

Reagent	One rxn	8 rxns
Binding Buffer AM9	99.25 µl	794 µl
1 M DTT	0.25 µl	2 µl
Protease Inhibitor Cocktail	0.5 µl	4 µl
Total Volume	**100 µl**	**800 µl**

3. Dilute the fragmented DNA in water if necessary. The HypoMethylCollector protocol can be performed on a large range of input DNA amounts (10 ng to 1 µg). We recommend 200 ng for the control Mse I digested human, male genomic DNA.

   Note 1: If using 200 ng - 1 µg of starting material, it is recommended to keep the DNA concentration high so that only 10 µl of DNA sample is used for each reaction. Following fragmentation, DNA can be precipitated and concentrated if necessary.

   Note 2: If the downstream application is high-throughput sequencing, please read the notes in Troubleshooting in Appendix D before beginning the binding reaction.

4. In this step, the Input DNAs that will be used in the final PCR step are prepared.

   a. **If performing real time PCR:** For the control genomic DNA provided in the kit, it is recommended that several Input DNA concentrations be run in triplicate. Input DNA should be tested at 0.01, 0.1, 1 and 10 ng/µl.

   b. **If performing endpoint PCR:** For the control genomic DNA provided in the kit, PCR analysis is performed for 36 cycles on 25 ng of control DNA. The control DNA (provided at 20 ng/µl) should be diluted to 5 ng/µl for use in Input PCR. This can be done by diluting the DNA 1/4 in dH_2O (*e.g.*, 5 µl of 20 ng/µl DNA + 15 µl dH_2O to make 5 ng/µl DNA). 5 µl of the 5 ng/µl DNA is used for Input PCR.

   Note: Customer sample Input DNA can be treated similarly. If your locus-specific PCR primers are efficient and PCR will be performed for 36 cycles, 25 ng of sample DNA can be used for the Input PCRs. However, PCR primer efficiency varies and you may want to try several amounts of Input DNA to be sure to obtain PCR products from reactions still in the linear phase of amplification.

5. Using the PCR tubes provided, fully resuspend magnetic beads by inverting, then aliquot a 10 µl slurry into each tube. If preparing more than 4 reactions, cap and re-invert the beads after every 4 aliquots. (**Note:** When working with magnetic beads, pipette gently.)

6. **Binding Reaction:** Add the remaining components in the order shown below to each PCR tube. Pipet the His-CXXC protein up and down several times to ensure homogeneity before use. Prepare a positive control reaction using the provided *Mse* I digested human, male genomic DNA. A negative control, no protein, reaction is also recommended.

Reagent	Sample (One rxn)	Positive Control	Negative Control
Magnetic beads	10 µl	10 µl	10 µl
Complete Binding Buffer	70 µl	70 µl	80 µl
His-CXXC (0.65 µg/µl)	10 µl	10 µl	-
Total Volume	**90 µl**	**90 µl**	**90 µl**

   Note: It is recommended to aliquot the provided His-CXXC protein into several small fractions to avoid multiple freeze/thaw cycles. Store at -20°C.

7. Pipet up and down to mix the components. Incubate at room temperature for 5 minutes.
8. Add fragmented genomic DNA. For samples and negative control, use 10 ng - 1 µg sample DNA in a final volume of 10 µl. For the positive control, use 10 µl of the provided *Mse* I digested human, male genomic DNA.

Reagent	Sample (One rxn)	Positive Control	Negative Control
Human genomic DNA control (20 ng/µl)	-	10 µl	-
Fragmented Genomic DNA (in a final volume of 10 µl)	from 10 ng - 1 µg	-	from 10 ng - 1 µg

8. Cap tubes and shake to mix thoroughly. Incubate on a rotisserie shaker for 30 minutes at room temperature.

Step 3: Wash beads

1. After the capture step is complete, spin the PCR tubes briefly and place tubes on a magnetic stand to pellet beads on the tube side. Carefully remove and SAVE the supernatant (unbound fraction) in a DNase-free 1.5 ml microcentrifuge tube. Set the unbound fraction aside at 4°C for DNA clean up in Step 5. To use the magnet provided in the kit, please see Appendix C.
2. Wash beads four times with 200 µl Binding Buffer AM8 (use Binding Buffer AM8 for wash steps regardless of binding buffer used to capture unmethylated CpGs). Pipette 4-5 times gently to resuspend.
    a. Place tubes on magnetic stand and allow beads to pellet on the side of the tube.
    b. Carefully remove the supernatant and any residual bubbles.
    c. Add 200 µl Binding Buffer AM8 and resuspend the pellet completely by pipetting several times. Ensure that the beads do not stick to the pipette tips. In most cases, the beads can be completely resuspended while the tubes are in the magnetic stand. However, depending on the strength of the magnet being used, it may be necessary to move the tubes to a separate rack before resuspending.
    d. Repeat steps a-c.
3. After the final wash, place tubes on magnetic stand and allow beads to settle to the side. Remove and discard supernatant without disturbing the beads.

Step 4: Recovery of unmethylated DNA fragments

1. Resuspend washed beads with 100 µl Elution Buffer AM3 by pipetting 5-6 times.
2. Place tubes in magnetic stand and allow beads to pellet onto tube sides.
3. Carefully transfer the supernatant to a fresh DNase-free 1.5 ml microcentrifuge tube.
4. Proceed to Step 5, DNA clean up, or else DNA can be stored at -20°C. If the DNA is stored at -20°C, it recommended to be reheated at 37°C for 10 minutes prior to use.

Step 5: DNA clean up

Prior to PCR amplification it is necessary to clean up the DNA. DNA clean up can be performed using one of the methods listed below on both the eluted hypomethylated fraction and the unbound fraction from Step 3, No. 1.

- **Purification columns** – use PCR clean up kits such as Active Motif's Chromatin IP DNA Purification Kit (Catalog No. 58002). Samples should be eluted in 50 µl volume, or other appropriate volume of water or buffer as needed for specific downstream applications.

  For microarray analysis, use MinElute PCR Purification Kit (Qiagen part no. 28004) and elute in 10 µl volume to obtain more concentrated DNA.

- **Phenol/chloroform extraction followed by ethanol precipitation** – follow the protocol listed below.

Phenol/Chloroform Extraction & Ethanol Precipitation

1. Add an equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1, v/v/v) to the eluted sample and, if desired, the unbound fraction from Step 2, No. 1.
2. Vortex the tube at maximum speed for 15 seconds.
3. Centrifuge the tube for 5 minutes at 12,000 x *g* at room temperature.
4. Carefully transfer the top, aqueous phase to clean microcentrifuge tube without collecting any of the lower organic phase or precipitate that may occur between the phases.
5. To each sample add:

   1 µl Glycogen (20 mg/ml) (included in the kit)

   1 sample volume of 5 M ammonium acetate

   2.5 sample volumes of 100% ethanol
6. Mix well and incubate at -80°C for at least 2 hours.
7. Centrifuge the tube for 20 minutes at 12,000 x *g*, 4°C.
8. Carefully discard the supernatant without disturbing the pellet.
9. Add 500 µl of cold 70% ethanol. Do not disturb the pellet.
10. Centrifuge the tube for 10 minutes at 12,000 x *g*, 4°C.
11. Carefully discard the supernatant without disturbing the pellet.
12. Air-dry the pellet for 5 minutes (do not completely dry the pellet).
13. Resuspend the DNA pellet in 50 µl sterile DNase-free water, or use other appropriate volumes of buffer or water as needed for specific downstream applications.
14. This eluted DNA can be used immediately in PCR or stored at -20°C. If the DNA is stored at -20°C, it will need to be reheated at 37°C for 10 minutes prior to use in PCR reactions.

References

1. Bird, A.P. (1986) *Nature*, 321: 209-213.
2. Illingworth, R. *et al*. (2008) *PLoS Bio*., 6: 0037-0051.
3. Henikoff, S. and Matzke, M.A. (1997) *Trends Genet*., 13: 293-295.
4. Wolffe, A.P. and Matzke, M.A. (1999) *Science*, 286: 481-486.
5. Reik, W. *et al.* (2001) *Science*, 293: 1089-1093.
6. Grewal, S.I. and Moazed, D. (2003) *Science*, 301: 798-802.
7. Antequera, F. and Bird, A. (1993) *Proc. Natl Acad. Sci. USA*, 90: 11995-11999.
8. Jones, P.A. and Baylin, S.B. (2002) *Nature Rev. Genet*., 3: 415-428.
9. Esteller, M. *et al.* (2001) *Cancer Res.*, 61: 3225-3229.
10. Feinberg, A.P. and Vogelstein, B. (1983) *Nature*, 301: 89-92.
11. Costello, J.F. *et al.* (2000) *Nat. Genet*., 24: 132-138.
12. Esteller, M. (2005) *J. Pathol.*, 205: 172-180.
13. Kalebic, T. (2003) *Ann. N Y Acad. Sci.*, 983: 278-285.
14. Ballestar, E. and Wolffe, A.P. (2001) *Eur. J. Biochem.*, 268: 1-6.
15. Nan, X. *et al.* (1993) *Nucleic Acids Res.*, 21: 4886-4892.
16. Hendrich, B. and Bird, A. (1998) *Mol. Cell. Biol.*, 18: 6538-6547.
17. Wade, P.A. *et al.* (1999) *Nat. Genet*., 23: 62-66.
18. Feng, Q. and Zhang, Y. (2001) *Genes Dev.*, 15: 827-832.
19. Magdinier, F. and Wolffe, A.P. (2001) *Proc. Natl Acad. Sci. USA*, 98: 4990-4995.
20. El-Osta, A. *et al.* (2002) *Mol. Cell. Biol*., 22: 1844-1857.
21. Fraga, M.F. *et al.* (2003) *Nucleic Acids Res*., 31, 1765-1774.
22. Jorgensen, H. *et al.* (2004) *Mol. Cell. Biol*., 24: 3387-3395.
23. Cross, S.H. *et al*. (1997) *Nat. Genet*., 16: 256-259.
24. Fujita, N. *et al*. (1999) *Mol. Cell. Biol.*, 19: 6415-6426.
25. Birke, M., *et al*. (2002) *Nucleic Acids Res*., 30: 958-965.
26. Lee, J.H., *et al.* (2001) *J. Biol. Chem*., 276: 44669-44676.
27. Acevedo, L.G. *et al*. (2011) *Epigenomics*, 3(1): 93-101.
28. Dalh, C. and Guldberg, P. (2003) *Biogerontology*, 4: 233-250.
29. Frommer, M. *et al.* (1992) *Proc. Natl Acad. Sci. USA*, 89: 1827-1831.
30. Weber, M. *et al.* (2005) *Nat. Genet*., 37: 853-832.

Appendix

Section A. Downstream Applications

The His-CXXC protein used in the HypoMethylCollector Kit can be used to selectively bind and enrich for unmethylated CpG dinucleotides. The amount of DNA recovered from the HypoMethylCollector Kit will depend on the global methylation status of the sample DNA. Usually only a small percentage of the starting material will be recovered, yet the recovered sample is specific for hypomethylated DNA fragments.

PCR Analysis

By amplifying a specific target site using PCR, it is possible to determine if the target region is predominately unmethylated. Alternatively, the same site can be compared across different sample types, but it will be necessary to run HypoMethylCollector on the same amount of each DNA sample and generate a separate standard curve for each sample.

Microarray Analysis

Researchers who are interested in the global changes to methylation pattern due to an experimental treatment rather than specific target analysis may prefer to analyze the enriched DNA by microarray. For an overview of this technology, please visit http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html.

HypoMethylCollector can be used prior to microarray analysis. The final elution of the CXXC pulled-down DNA should be passed through a DNA clean-up column, such as Qiagen MinElute PCR Purification Kit (part no. 28004), to clean the DNA and minimize the final recovery volume for a more concentrated DNA sample. To perform the microarray analysis, microgram quantities of DNA will be needed. Since the amount of DNA recovered from the HypoMethylCollector Kit depends on the global methylation status of the sample, it may be necessary to pool multiple samples together or to perform whole genome amplification on the recovered material in order to obtain microgram quantities. Once microgram quantities of DNA have been obtained, the HypoMethylCollector DNA can be labeled with Cy5 and the input DNA can be labeled with Cy3 for hybridization to the microarray.

Whole genome amplification

Active Motif's GenoMatrix™ Whole Genome Amplification Kit (Catalog No. 58001) has been successfully validated for use with the GC-rich DNA from the HypoMethylCollector Kit and shown to maintain the sequence representation of the starting material. It is recommended to start with 500 ng - 1 µg of fragmented DNA per binding reaction. Alternatively, multiple small reactions can be pooled together. 10 ng is the minimum starting material required for the GenoMatrix™ Whole Genome Amplification Kit.

To amplify DNA without using the GenoMatrix™ Whole Genome Amplification Kit, a Ligated Mediated PCR will need to be performed. This will involve blunt ending the enriched, purified DNA, ligating blunt adaptors and PCR amplifying for 14-20 cycles. Amplicons produced from this method

are generally between 300-500 bp due to a bias for smaller PCR products. The ligation reaction also has a tendency to be less efficient. However, after amplification you should obtain the microgram quantities of DNA needed for microarray analysis.

Non-amplification

If whole genome amplification will not be performed, multiple HypoMethylCollector reactions will need to be combined in order to achieve microgram yields. It may be necessary to combine 10 or more separate binding reactions from the same sample material.

Section B. PCR Analysis

PCR Primer Design

HypoMethylCollector includes PCR primers for use with the provided *Mse* I digested human, male genomic DNA. If possible, real time PCR is recommended for analysis of DNA isolated with HypoMethylCollector. To design primers specific to the CpG region of interest in your sample, please follow the recommendations below.

Primer design considerations

i. Primers should flank the CpG region of interest and produce an amplicon between 100-350 bp in length for end point PCR or an amplicon of 100-150 bp for real time PCR. The CpG region needs to contain at least five CpGs.

ii. Each primer should be approximately 18-22 nucleotides long, contain 50% GC content and have a T_m between 55°C - 60°C.

iii. Restriction-digested DNA: PCR primer pairs should amplify a restriction fragment (or portion of a restriction fragment) that contains a CpG-rich region of interest. Each amplicon must also be free of internal sites for the restriction enzyme.

iv. Sonicated DNA: PCR primers should flank the CpG-containing region of interest and the amplicon should not contain any CpG-dinucleotides that are outside of this region. This will minimize amplification of fragments that are isolated as a result of CpGs that are near, but not within, the CpG-rich region of interest.

v. PCR primers should be designed with the aid of a reliable primer design computer program (*e.g.*, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). Due to the technical limitations of PCR, it is sometimes necessary to design more than one primer pair for a given fragment of interest.

vi. Potential primer pairs can be evaluated via computer simulation using a program such as UCSC Genome Browser (http://genome.ucsc.edu/) to ensure the primers selected will produce a single amplicon in a CpG region of the species being amplified.

Determining Primer Efficiency

It is advised to determine the efficiency of the PCR primers being used. Primers with an efficiency less than 90% will have poor reproducibility. To calculate primer efficiency:

$$\text{Primer efficiency (\%)} = [10^{(-1/\text{slope})}-1] \times 100\%$$

To obtain the slope value, follow the instructions for generating and graphing a standard curve in the Data Analysis and Use of Input DNAs section on page 21. Use the slope of the plotted standard curve in the primer efficiency equation above.

Endpoint PCR Analysis

A typical endpoint PCR protocol example follows below. This protocol was optimized for the control samples. For each new set of primers amplifying the promoter region of interest, the PCR conditions have to be optimized carefully (optimal T_m, number of cycles, *etc.*).

1. For one PCR reaction:

Reagent	One rxn
Sterile water	9.8 µl
10X PCR Buffer	2.5 µl
10X PCR compatible loading dye	2.5 µl
dNTP mixture (5 mM each dNTP)	1 µl
Forward Primer* (5 pmol/µl)	2 µl
Reverse Primer* (5 pmol/µl)	2 µl
Taq (5 U/µl)	0.2 µl
DNA sample (eluted, unbound or Input)	5 µl
Total Volume	**25 µl**

* The provided PCR Primer Mixes contain Forward and Reverse primers for use with the provided control DNA. Use 4 µl of this mix in the typical PCR protocol described above.

2. Place tubes in a PCR thermocycler and program as below:

   94°C for 3 minutes
   (94°C for 20 seconds, 60°C for 30 seconds, 72°C for 30 seconds) for 36 cycles
   Hold at 4°C

3. Endpoint PCR can by analyzed by agarose gel electrophoresis. Run reactions by loading 10 µl from each of the PCRs on a thin 3% agarose gel at 125V for 50 minutes in parallel with an appropriate DNA ladder. Post-stain the gel with 1 µg/ml ethidium bromide in 1X TAE buffer for 20 minutes. Observe gel under UV.

   GAPDH Glyceraldehyde-3-phosphate dehydrogenase should be unmethylated in the control human, male genomic DNA and is expected to produce a 69 base pair PCR product in the eluted DNA fraction. There should be a strong band in the eluted fraction and a faint/no band in the unbound fraction.

   Xist X inactive specific transcript is a methylated promoter in human male genomic DNA, but is non-methylated in females. The control human, male genomic DNA should be methylated at this locus and is expected to produce a PCR product for the 178 base pair region amplified by included PCR primer mix in the unbound fraction. There should be a strong band in the unbound fraction and a faint/no band in the eluted fraction.

Real Time PCR Analysis

This is an example PCR reaction. Please follow the specific instructions for your real time PCR instrument.

1. For one PCR Reaction:

Reagent	10 µl PCR reaction	20 µl PCR reaction
Fast SYBR Green master mix	5 µl	10 µl
Forward primer* (5 pmol/µl)	0.5 µl	1 µl
Reverse primer* (5 pmol/µl)	0.5 µl	1 µl
Sterile water	1 µl	3 µl
DNA sample (eluted, unbound or Input)	3 µl	5 µl
Total volume	**10 µl**	**20 µl**

* The provided PCR Primer Mixes contain Forward and Reverse primers for use with the provided control DNA. Use 1 µl of the PCR Primer Mix in the 10 µl reaction or 2 µl of the PCR Primer Mix in the 20 µl reaction.

Note: It is recommended to prepare triplicates of each sample and Input reaction. Input DNA should be tested at 0.01, 0.1, 1 and 10 ng/µl to obtain a standard curve.

2. Place tubes in a Real Time PCR instrument and program as below. The amplification conditions should be optimized for each target locus and PCR instrument. A suggested starting point is:

   95°C for 20 seconds
   (95°C for 3 seconds, 60°C for 30 seconds) for 40 cycles

3. Analyze the results. Data analysis varies depending on the instrument used. Obtain the standard curve from the Input samples. Use the standard curve to quantify the DNA in each sample.

   GAPDH Glyceraldehyde-3-phosphate dehydrogenase should be unmethylated in the control human, male genomic DNA and is expected to amplify early in the eluted fraction.

   Xist X inactive specific transcript is a methylated promoter in human male genomic DNA, but is non-methylated in females. The control human, male genomic DNA should be methylated at this locus and will amplify early in the unbound fraction.

Data Analysis and Use of Input DNAs

DNA isolated using HypoMethylCollector is usually analyzed by PCR amplification of the loci of interest. However, if the goal is to compare the methylation status of particular loci in different DNA samples, it is essential that HypoMethylCollector be performed on the same amount of each DNA sample. Thus, DNA samples should be carefully quantified before use. In addition, Input DNA should be prepared for each of the different DNA samples (see Step 2. No. 4 in the Protocol) to clearly indicate the relative concentrations of the DNA samples.

For real time PCR, generating a standard curve using the input DNA enables accurate determination of the enriched DNA concentration.

1. Produce a standard curve at 10, 1, 0.1 and 0.01 ng/µl in triplicate using the Input DNA from Step 2, No. 4. We recommend running a standard curve every time HypoMethylCollector is performed. However, if the primer efficiency has been determined to be greater than 90% according to the calculations on page 18, the standard curve can be generated once and stored for future use with the same DNA sample.
2. Run each sample with the appropriate DNA standard (*i.e.* prepare a separate standard curve for each cell line or species tested).
3. Every gene will generate a different amplification profile. CT = Threshold Cycle or cycle number where the signal exceeds the background threshold level. CT values should be plotted for each gene to create a linear regression plot.
4. Plot CT versus log DNA concentration. See Figure 4 below.

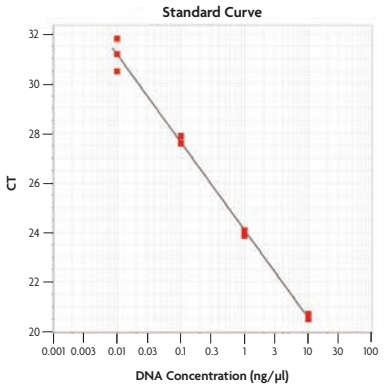


Figure 4: Example standard curve linear regression plot.

A standard curve for the human, male genomic DNA is provided as a reference only. Input DNA was tested in triplicate and plotted against the CT value. A new standard curve should be generated each time the assay is performed.

5. Using the CT value of the sample, extrapolate the DNA concentration of the sample DNA using the standard curve plot. To determine the amount of enriched DNA in the sample simply

multiply the DNA concentration by the volume of enriched DNA.

6. Calculate the percent enrichment. Use the sample DNA quantity calculated above and compare it with the amount of DNA used in the initial binding reaction (Step 2, No. 8).

$$\textbf{Enrichment}\ (\%) = \frac{\text{Amount of enriched Sample DNA material}}{\text{Amount of fragmented DNA used in the binding reaction}} \times 100\%$$

7. *Optional:* Calculate the fold enrichment of methylated DNA. If the unbound material was collected in Step 3, No. 1 and purified for analysis in PCR, the eluted samples can be compared to the unbound samples for the same locus in order to determine the fold enrichment.

$$\textbf{Fold enrichment} = \frac{\text{Sample DNA concentration in the eluted fraction}}{\text{Sample DNA concentration in the unbound fraction}}$$

Section C. Use of Magnetic Beads and Included Bar Magnet

Caution: The included neodymium bar magnet is extremely powerful and is easily broken if handled incorrectly.

1. The magnet should be stored in the provided tube.
2. Be careful when working near metal objects or surfaces. A free magnet will jump great distances onto nearby metal surfaces with surprising speed. This can break the magnet.
3. If the magnet becomes attached to a flat metal surface, it should be removed by sliding it off the edge of surface. The magnet may be broken if you attempt to pull one end away from the metal.

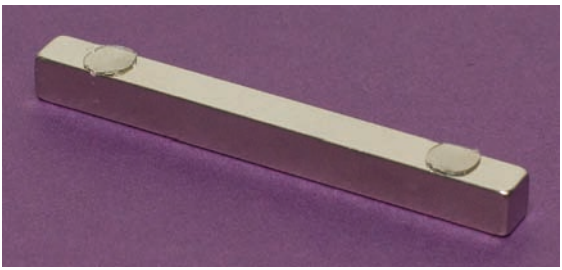
Assembly of Magnetic Stands

The provided Mini Glue Dots can be used to attach the bar magnet to an empty tip box to create an effective magnet stand.

Creating a magnetic stand for 8-well PCR strips:

Note: 8-well strip tubes for use with standard 96-well PCR cyclers are appropriate.

1. Remove the covering tape from one side of two glue dots.
2. Place a strip of PCR tubes in the wells of an empty tip box (200 µl tips) and place the magnet directly against the tubes. This is the way the magnet will be positioned when the glue dots are used to affix it to the box.
3. Attach the glue dots on the bar magnet (the uncovered face of the dot is placed on the magnet) as shown below.



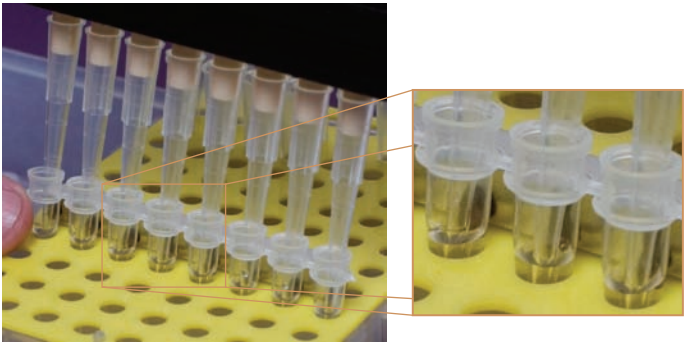
4. Remove the covering tape from the exposed side of the glue dots. Fix the magnet to the tip box so that it is against the PCR tubes. The magnetic stand is now ready for use.

Note: Familiarize yourself with using the magnetic stand before performing with PCR tubes for the first time. Add 5 µl of magnetic beads to 100 µl Binding Buffer AM8 in one tube of an 8-well strip of PCR tubes. Use this tube with the assembled bar magnet stand to become familiar with use of the beads and magnet. It is difficult

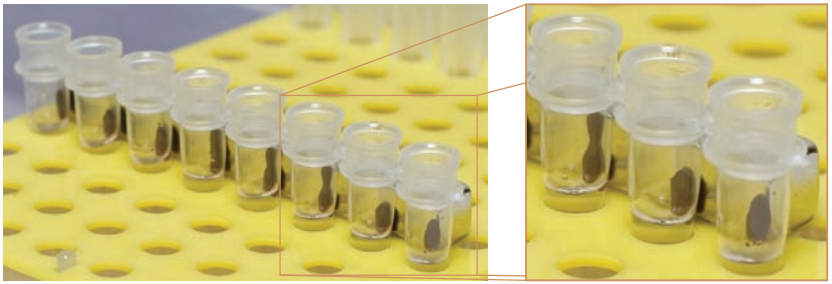
to resuspend the beads if the tubes are directly adjacent to the magnet, so it is usually best to move the tubes away from the magnet for resuspension.

Washing should be performed as follows:

a. Place the tubes in the rack against the magnet and allow the beads to be pinned to the side of the tube, as shown below.



b. Remove supernatant with a 200 µl pipetteman or a 200 µl eight-channel pipetteman.



c. Move the tube strip into a row that is not adjacent to the magnet.

d. Add wash buffer and pipette up and down to fully resuspend the beads. Ensure that a minimal amount of beads cling to the tips when the resuspension is complete.

e. Repeat steps a-d until desired washing steps are complete.

Centrifugation of 8-well PCR strip tubes:

When working with 8-well PCR strip tubes, it may be desirable to centrifuge the tubes to collect the liquid and beads from the inside of the caps. This is easily accomplished in a centrifuge fitted with adaptors for spinning microtiter plates. In this case, a standard 96-well plate can be placed in the adaptor to hold the tubes in place. Take care to ensure the rotor is balanced (*e.g.*, place a microtiter plate and tubes of appropriate mass in the rotor's opposing 96-well plate adaptor). Spin the plates briefly to let the rotor reach a speed of 1000 x *g* before allowing the rotor to stop.

Section D. Troubleshooting Guide

Problem/question	Recommendation
The target DNA fragment has less than 6 non-methylated CpGs.	The provided Binding Buffer AM8 is optimal for efficient capture of DNA fragments that have six or more non-methylated CpGs. For fragments with less than 6, we recommend using Binding Buffer AM9 (included in kit).
DNA starting material	HypoMethylCollector can be used with 10 ng - 1 µg of fragmented DNA. For capturing large amounts of DNA (200 ng - 1 µg) it is recommended to keep the DNA concentration high so that only 10 µl of DNA sample is used for each reaction. Following fragmentation, DNA can be precipitated and concentrated if necessary.
PCR amplification	It has been determined that using a hot-start polymerase (*i.e.* Phusion™ from NEB) instead of a classic *Taq* polymerase may also increase the sensitivity of the assay.
	Selection of an appropriate qPCR master mix is important to achieve good amplification. Since all commercially available mixes have different compositions regarding chemical enhancers or inhibitors for non-specific amplification, results may vary depending on the master mix used.
Storage of DNA	Once DNA is prepared using HypoMethylCollector, samples may be stored at -20°C prior to PCR analysis. However, we recommend heating the frozen material to 37°C for 10 minutes before use in PCR, as heat-treatment releases any DNA bound to the tube during storage.
Can I use 3 M sodium acetate, pH 5.2 instead of 5 M ammonium acetate in the ethanol precipitation?	Yes, 3 M sodium acetate, pH 5.2 can be used at 1/10th sample volume along with 2 sample volumes of 100% ethanol during the precipitation step. However, we have noticed that the ammonium acetate had better yield of recovery than the sodium acetate in a direct comparison of several samples.
Should I use Restriction Digest or Sonication to fragment my DNA?	Restriction Digest is very precise and reproducible, however, the DNA must be well purified and analysis of several loci would also require use of different enzymes. In addition, the region of interest may not be flanked by suitable restriction sites and SNPs between different cell types may confound results. In contrast, Sonication is random, which enables analysis of many loci simultaneously (microarray), but it may not be possible to shear DNA small enough to isolate CpG islands of interest. In addition, results may vary from shearing to shearing depending on sonicator used. Also it is difficult to prepare DNA from a small number of cells.
Heat inactivation or removal of restriction enzyme used to fragment DNA	After restriction digest, we recommend that samples be treated for 20 minutes at 65°C. Some enzymes (such as *Mse* I) will be inactivated by this treatment, while those that are not will be forced off the DNA. In most cases (even when using enzymes that are not heat-inactivated), DNA treated in this fashion should be suitable for use in the HypoMethylCollector protocol. In some situations (*e.g.*, when the DNA used in a digest is contaminated with cellular proteins or when a large amount of restriction enzyme is required for the digest) it may be desirable to purify the digested DNA by purification columns or through phenol extraction/ethanol precipitation.
10X PCR Loading Dye	If PCR is performed using the 10X PCR Loading Dye provided, it is not necessary to add additional loading dye to the samples before running samples on agarose gel.
High-throughput sequencing analysis	The His-CXXC protein stock may contain some trace amounts of bacterial DNA. If the desired downstream application is high-throughput sequencing analysis, we recommend pre-treatment of the required amount of His-CXXC protein with DNase I for 2 hours at room temperature on a rolling shaker. Use 0.5 µl DNase I (1 U/µl) and 1 µl of 10X DNase I buffer for every 10 µl of His-CXXC protein. Following the DNase treatment, the protein is ready for use in the binding reaction. After the 5 minute binding reaction, place the tubes on the magnetic stand to pellet the beads (see Appendix Section A for instructions on using the magnetic stand). Wash the bead pellet twice with 200 µl Binding Buffer AM8 in order to remove the DNase I. Resuspend beads in 80 µl of either complete Binding Buffer AM8 or AM9 depending on the number of non-methylated CpGs per DNA fragment. Proceed with the protocol at Step 1, No. 7 for the addition of fragmented DNA.

Section E. Related Products

DNA Methylation	Format	Catalog No.
MethylDetector™	50 rxns	55001
MethylCollector™ Ultra	30 rxns	55005
HypoMethylCollector™	30 rxns	55004
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007
Methylated DNA Standards	3 x 2.5 µg	55008
hMeDIP	10 rxns	55010

Whole Genome Amplification	Format	Catalog No.
GenoMatrix™ Whole Genome Amplification Kit	50 rxns	58001

Antibodies	Application	Format	Catalog No.
DNMT1 mouse mAb	ChIP, IHC, IP, WB	100 µg	39204
DNMT2 rabbit pAb	WB	100 µg	39205
DNMT3A mouse mAb	ChIP, IF, IHC, WB	100 µg	39206
DNMT3B mouse mAb	ChIP, IF, IP, WB	100 µg	39207
MBD1 mouse mAb	WB	100 µg	39215
MBD2 mouse mAb	WB	100 µg	40965
MBD3 mouse mAb	WB	100 µg	39216
MBD4 mouse mAb	WB	100 µg	39217
MeCP2 rabbit pAb	WB	100 µg	39218

Recombinant Methylated Histones	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Active Motif also offers a growing list of application validated antibodies, including antibodies for histones and histone modifications, transcription factor antibodies, DNA methylation-related

antibodies and ChIP validated antibodies. For a complete list go to **www.activemotif.com/abs**

Histone Purification	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	20 rxns	40026

Chromatin Assembly	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Co-Immunoprecipitation	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rxns	54001
Universal Magnetic Co-IP Kit	25 rxns	54002

SUMOylation	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™	25 rxns	53001
ChIP-IT™ w/o controls	25 rxns	53004
ChIP-IT™ Shearing Kit	10 rxns	53002
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Enzymatic w/o controls	25 rxns	53007
Enzymatic Shearing Kit	10 rxns	53005
Salmon Sperm DNA/Protein G agarose	25 rxns	53003
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

Control Acid Extracts	Format	Catalog No.
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201
HeLa acid extract (Sodium Butyrate treated)	100 µg	36202

HeLa acid extract (Etoposide treated)	100 µg	36203
HeLa acid extract (Anacardic acid treated)	100 µg	36204

Transcription Factor ELISAs	Format	Catalog No.
TransAM™ AML-1/Runx1	1 x 96-well plate	47396
TransAM™ AML-3/Runx2	1 x 96-well plate	44496
TransAM™ AP-1 Family	2 x 96-well plates	44296
TransAM™ AP-1 c-Fos	1 x 96-well plate	44096
TransAM™ AP-1 c-Jun	1 x 96-well plate	46096
TransAM™ AP-1 FosB	1 x 96-well plate	45096
TransAM™ AP-1 JunD	1 x 96-well plate	43496
TransAM™ ATF-2	1 x 96-well plate	42396
TransAM™ c-Myc	1 x 96-well plate	43396
TransAM™ C/EBP α/β	1 x 96-well plate	44196
TransAM™ CREB	1 x 96-well plate	42096
TransAM™ pCREB	1 x 96-well plate	43096
TransAM™ Elk-1	1 x 96-well plate	44396
TransAM™ ER	1 x 96-well plate	41396
TransAM™ FKHR (FOXO1/4)	1 x 96-well plate	46396
TransAM™ GATA Family	2 x 96-well plates	48296
TransAM™ GATA-4	1 x 96-well plate	46496
TransAM™ GR	1 x 96-well plate	45496
TransAM™ HIF-1	1 x 96-well plate	47096
TransAM™ HNF Family	2 x 96-well plates	46296
TransAM™ HNF-1	1 x 96-well plate	46196
TransAM™ IRF-3 (Human)	1 x 96-well plate	48396
TransAM™ IRF-3 (Mouse)	1 x 96-well plate	48496
TransAM™ IRF-7	1 x 96-well plate	50196
TransAM™ MAPK Family	2 x 96-well plates	47296
TransAM™ MEF2	1 x 96-well plate	43196
TransAM™ MyoD	1 x 96-well plate	47196
TransAM™ NF-YA	1 x 96-well plate	40396
TransAM™ NFATc1	1 x 96-well plate	40296
TransAM™ NFκB Family	2 x 96-well plates	43296
TransAM™ Flexi NFκB Family	2 x 96-well plates	43298
TransAM™ NFκB p50	1 x 96-well plate	41096
TransAM™ NFκB p50 Chemi	1 x 96-well plate	41097
TransAM™ Flexi NFκB p50	1 x 96-well plate	41098
TransAM™ NFκB p52	1 x 96-well plate	48196
TransAM™ NFκB p52 Chemi	1 x 96-well plate	48197
TransAM™ NFκB p65	1 x 96-well plate	40096
TransAM™ NFκB p65 Chemi	1 x 96-well plate	40097
TransAM™ Flexi NFκB p65	1 x 96-well plate	40098
TransAM™ Nrf2	1 x 96-well plate	50296
TransAM™ Oct-4	1 x 96-well plate	42496
TransAM™ p53	1 x 96-well plate	41196
TransAM™ PPARγ	1 x 96-well plate	40196
TransAM™ Sp1	1 x 96-well plate	41296
TransAM™ Sp1/Sp3	1 x 96-well plate	40496
TransAM™ STAT Family	2 x 96-well plates	42296
TransAM™ STAT3	1 x 96-well plate	45196
TransAM™ T-bet	1 x 96-well plate	51396

For a complete, up-to-date list of available TransAM™ Kits, please visit **www.activemotif.com/transam**

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

Active Motif North America
1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008
USA
Toll Free: 877 222 9543
Telephone: 760 431 1263
Fax: 760 431 1351
E-mail: tech_service@activemotif.com

Active Motif Europe
Avenue Reine Astrid, 92
B-1310 La Hulpe, Belgium
UK Free Phone: 0800 169 31 47
France Free Phone: 0800 90 99 79
Germany Free Phone: 0800 181 99 10
Telephone: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
E-mail: eurotech@activemotif.com

Active Motif Japan
Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Telephone: +81 3 5225 3638
Fax: +81 3 5261 8733
E-mail: japantech@activemotif.com

Visit Active Motif on the worldwide web at **http://www.activemotif.com**

At this site:

- Read about who we are, where we are, and what we do
- Review data supporting our products and the latest updates
- Enter your name into our mailing list to receive our catalog, *MotifVations* newsletter and notification of our upcoming products
- Share your ideas and results with us
- View our job opportunities

Don't forget to bookmark our site for easy reference!