

Methylated DNA Immunoprecipitation (MeDIP) (PROT33)



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Introduction

Methylation of cytosines can mediate epigenetic gene silencing and is the most prominent DNA modification in eukaryotes. MeDIP is an immunocapturing approach to enrich DNA that is methylated. The principle is that genomic DNA is randomly sheared by sonication and immunoprecipitated with an antibody that specifically recognizes 5-methylcytidine (5mC) ([Figure 1](#)). This protocol has been used to generate comprehensive DNA methylation profiles on a genome scale in mammals and plants[1-3], and to identify abnormally methylated genes in cancer cells [1].

Procedure

Preparation of genomic DNA

The MeDIP protocol has been successfully used with genomic DNA from various organisms that contain methylated cytosines in their genome (human, mouse, *Arabidopsis thaliana*, *Neurospora crassa*).

1. Resuspend the cell pellet or the homogenized tissue in 300 µl TE in a 2 ml eppendorf tube (the volume should be increased for big cell pellets or tissue samples)
2. Add 300 µl [Lysis buffer](#) containing 20 µl proteinase K (10 mg/ml stock) ([note 1](#))
3. Incubate at 55°C for at least 5 hours
4. Extract with 1 volume phenol (600 µl)
5. Extract with 1 volume chloroform (600 µl)
6. Precipitate the DNA with 2 volumes ethanol 75 mM NaAcetate (1.2 ml)
7. Resuspend the DNA pellet in TE containing 20 µg/ml RNase A ([note 2](#))

Sonication of genomic DNA

Genomic DNA is randomly sheared by sonication to generate fragments between 300 and 1000 bp. Genomic DNA can also be fragmented with restriction enzymes like *A**l**u**I*, but it is not recommended for unbiased microarray studies. The sonication efficiency varies with DNA concentration, sonicator settings and size and quality of the sonication tip, therefore it is recommended to systematically check the size of the sheared DNA to ensure equal sonication between experiments.

1. Dilute the genomic DNA in TE in a 1.5 ml eppendorf tube (10-20 µg DNA in 400 µl TE, 40-60 µg DNA in 700 µl TE)
2. Sonicate 5 times 10 seconds (BRANSON digital Sonifier model 450, used with the tapered Microtip, amplitude 20%), with 1 minute intervals between pulses (keep the tube on ice during the sonication)
3. Load 5 µl on an agarose gel to check the size of the DNA (mean size should be 300-1000 bp) ([Figure 2](#))
4. If necessary, sonicate one or two additional pulses until the size of the DNA is 300-1000 bp
5. Precipitate the sonicated DNA with 400 mM NaCl, glycogen (1 µl) and 2 volumes 100% ethanol
6. Resuspend the DNA pellet in water and measure DNA concentration

Immunoprecipitation of methylated DNA (MeDIP)

The sonicated DNA is then immunoprecipitated with a monoclonal antibody against 5-methylcytidine (5mC) [4] (Eurogentec #BI-MECY-1000). A portion of the sonicated DNA should be left untreated to serve as input control.

1. Dilute 4 µg ([note 3](#)) of sonicated DNA in 450 µl TE
2. Denature for 10 minutes in boiling water and immediately cool on ice for 10 minutes
3. Add 51 µl of [10x IP buffer](#)
4. Add 10 µl of [5mC Antibody](#) ([note 4](#))
5. Incubate 2 hours at 4°C with overhead shaking
6. Pre-wash 40 µl of Dynabeads with 800 µl PBS-BSA 0.1% for 5 minutes at RT with shaking ([comment 1](#))
7. Collect the beads with a magnetic rack and repeat wash with 800 µl PBS-BSA 0.1%
8. Collect the beads with a magnetic rack and resuspend in 40 µl of 1x IP buffer
9. Add Dynabeads to the sample ([note 5](#))
10. Incubate 2 hours at 4°C with overhead shaking
11. Collect the beads with a magnetic rack and wash with 700 µl 1x IP buffer for 10 minutes at RT with shaking
12. Repeat wash with 700 µl 1x IP buffer twice
13. Collect the beads with a magnetic rack and resuspend in 250 µl [Proteinase K digestion buffer](#)
14. Add 7 µl proteinase K (10 mg/ml stock)
15. Incubate 3 hours at 50°C (use a shaking heating block 800rpm to prevent sedimentation of the beads) ([comment 2](#))
16. Extract with 1 volume phenol (250 µl)
17. Extract with 1 volume chloroform (250 µl)
18. Precipitate the DNA with 400 mM NaCl (20 µl NaCl 5M), glycogen (1 µl) and 2 volumes 100% ethanol (500 µl)
19. Resuspend the DNA pellet in 60 µl TE and keep at -20°C ([note 6](#))

Analysis by PCR and microarrays

Enrichments in the MeDIP fraction can be measured by PCR or by microarray analysis ([Figure 1](#)). Keep in mind that CpGs are unequally distributed in mammalian genomes and that the enrichment in the MeDIP fraction depends both on the methylation status of the target sequence and the number of CpGs it contains [2]. Therefore a low enrichment can reflect an unmethylated state or the absence of CpGs.

For PCR or real-time PCR, use 20 ng of total input DNA and 2 µl of MeDIP DNA. Enrichments in the MeDIP fraction are calculated relative to an unmethylated control, which is typically an unmethylated CpG island promoter from a housekeeping gene. For genome-wide analyses, input and MeDIP fractions are differentially labeled with Cy3 and Cy5 and co-hybridized to microarrays as a two-color experiment. The methylation level is measured as the intensity ratio of immunoprecipitated to input DNA ([Figure 1](#)). For the MeDIP fraction, DNA from parallel MeDIPs can be pooled. Alternatively, if an amplification step is needed, we recommend to use the Whole Genome Amplification (WGA) protocol (see NoE protocol PROT30) that can be applied to single-stranded DNA samples, with the caveat that amplification might not efficiently work for CpG-rich sequences ([comment 3](#)).

Materials & Reagents

5mC Antibody	We use the mouse monoclonal 5-methylcytidine antibody from Eurogentec (#BI-MECY-1000). A similar antibody is available from other companies (Calbiochem, Diagenode). The antibody should be stored as 20 µl aliquots at -20°C.
Magnetic beads	Dynabeads M-280 Sheep anti-mouse IgG (DynaL Biotech #112.01)
Lysis buffer	20 mM Tris pH 8.0 4 mM EDTA 20 mM NaCl 1 % SDS
PBS-BSA 0.1%	9 ml PBS 1 ml BSA 10 mg/ml stock
10x IP buffer	100 mM Na-Phosphate pH 7.0 1.4 M NaCl 0.5 % Triton X-100 (10x and 1x IP buffer can be kept at RT for several weeks)
1 M Na-Phosphate pH 7.0	39 ml 2 M monobasic sodium phosphate (NaH ₂ PO ₄) (276g/L) 61 ml 2 M dibasic sodium phosphate (Na ₂ HPO ₄)(284 g/L) 100 ml H ₂ O
Proteinase K digestion buffer	50 mM Tris pH 8.0 10 mM EDTA 0.5 % SDS

Author Notes

1. Due to the viscosity of the solution after cells lysis, it is recommended to add the Proteinase K to the [Lysis buffer](#) before mixing it with the sample.
2. It is important to remove RNAs efficiently, as the antibody also recognizes 5-methylcytidine in the context of RNA molecules.

3. I recommend to use 4 μg , however smaller amounts down to 1 μg can also be used.
4. Increasing the amount of antibody results in higher background. Using less antibody can give higher enrichment of methylated CpG islands over unmethylated sequences, but ultimately it also leads to loss of enrichments of methylated CpG-poor targets.
5. The amount of Dynabeads can be increased to recover more DNA, but it also results in higher background.
6. With the described conditions, the MeDIP procedure generally yields 5% of the original total DNA in mammalian cells (i.e. 200 ng of methylated DNA starting from 4 μg total DNA).

Reviewer Comments

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1. I found pre-washing the beads to be unnecessary.
2. I add proteinase K directly to the IP buffer and incubate for 30 min.
3. I have used the T7-based amplification protocol developed by the Bernstein lab (Liu et al, BMC Genomics, 2003) with a slight modification (ref 3) and compared the results to unamplified DNA. With the caveat that I always label with random primers, so a fair amount of amplification takes place during labeling, I haven't observed any biases caused by the T7-based method.

Figures

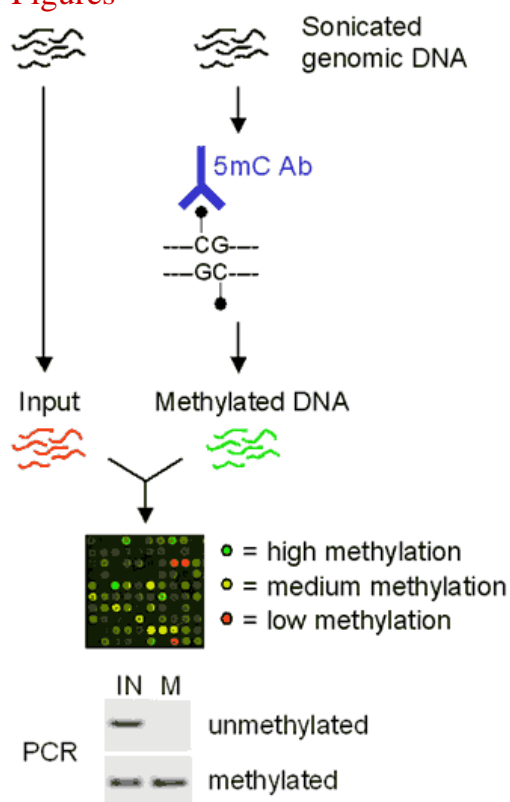


Figure 1.

Principle of MeDIP (Methylated DNA Immunoprecipitation). Total genomic DNA is sonicated and methylated DNA is immunoprecipitated with an antibody directed against 5-methylcytosine (5mC). Input DNA (IN) and methylated DNA (M) can be differentially labeled with Cy5 (red) and Cy3 (green) and co-hybridized as a two-color experiment on microarrays, or used for single-gene analysis by PCR.

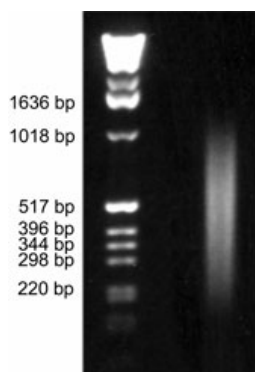


Figure 2.

The gel represents sonicated genomic DNA run on a 2% agarose gel stained with ethidium bromide. Most of the sheared fragments have a size between 300 and 1000 bp.

References

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4. Reynaud C, Bruno C, Boullanger P, Grange J, Barbesti S, Niveleau A: Monitoring of urinary excretion of modified nucleosides in cancer patients using a set of six monoclonal antibodies. *Cancer Lett* 1992, **61**:255-262.

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Artur Muradyan :

Posted 98 days ago

It would be better first to incubate Ab with Dynabeads, then add the Ab-beads complex on DNA.

< 1 >



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