Supporting Information

Qualitatively and Quantitatively Detection of Methylation at CpG Sites by Fluorescein-dGTP Incorporated Asymmetric PCR Assay Strategy

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Materials and Measurements: DNA templates and primers were purchased from Invitrogen (Shanghai, China). Hotstart Taq polymerase was obtained from Thermo. Fluorescein-labeled dGTP was purchased from Perkin Elmer. Non-denaturing polyacrylamide gel electrophoresis products were scanned with Pharos FX Molecular imager (Bio-Rad, USA).

Bisulfite treatment: Bisulfite conversion were performed according to the previous literature (see reference 8 in the text). About 1 μg DNA in a final volume of 50 μL was denatured by 0.2 M NaOH at 42 °C for 30 min. Then 30 μL of 10 mM freshly prepared hydroquinone was added to give a yellow solution, followed by the addition of 520 μl sodium bisulfite (3.6 M, pH = 5.0, freshly prepared). The samples were mixed gently and covered by 200μL mineral oil before incubated in 50°C for 16 h. The mixture were desalted by Millipore membranes for three times, followed by ethanol precipitation. The final modified DNA was dissolved in 30 μL ddH_20.

Primer design and PCR amplification: The primers used in asymmetry PCR do not contain any CpG sites, so that all the template DNA with different methylation status can be successfully amplified. The PCR mixture contains 1 × PCR reaction buffer, 1μL dATP, dTTP and dCTP (2 mM), 0.5μL dGTP, 1.5μL fluorescein-labeled dGTP (the final concentrations of four bases were kept identical), 2.5 U Hotstart Taq polymerase, 0.5 μL forward primer (1 μM), 5 μL reverse primer (10 μM) and bisulfite-treated DNA (120 ng for genomic DNA and 20 ng for 76-mer template DNA) in a final volume of 20 μL. The asymmetry PCR amplification were carried out under the following conditions: 95 °C for 10 min, and then 35 cycles of PCR at 94 °C for 30s, 48 °C for 30s (for 76-mer template DNA amplification, the annealing temperature for 218bp-E-cadherin promoter is 54 °C) and 72 °C for 30s, followed by final extension for 5 min at 72 °C.

PAGE analysis and fluorescence measurement: All the PCR products were loaded into a 12% non- polyacrylamide gel for electrophoresis in 1 × TBE buffer. The gel was then analysed using the Pharos FX Molecular Imager (Bio-Rad, USA). As for fluorescence measurement, fluorescein-labeled dGTP was separated from the PCR products firstly by using Millipore membranes three times.

Preparation of Genomic DNA: All the cancer cell lines used were cultured in RPMI-1640 supplemented with 10% fetal bovine serum and 5% penicillin-streptomycin. Genomic DNA were extracted and purified by CWBIO Genomic DNA kit according to the manufacturer's instruction.

5-aza-2'-deoxycytidine (DAC) treatment: 1×10^5 cells were treated with 10 μM of 5-aza-2'-deoxycytidine (DAC) for different times (0 h, 20 h, 40 h and 64 h). This concentration of DAC was chosen because it can provide efficient demethylation effect with minimal cytotoxicity. The corresponding genomic DNA were obtained by the same method

described before.

Table **S1**: DNA templates and primers used in this study

Name	Sequences (5'-3')
Template-C	CCTCACCATCTCAACCAATATTATATTACGCGTATAACGCGTATTGCGCGCTATAATATTGAGGGAGAAGTGGTGA
Template-1m	CCTCACCATCTCAACCAATATTATATTACGCGTATAA^{m}CGCGTATTGCGCGCTATAATATTGAGGGAGAAGTGGTGA
Template-2m	CCTCACCATCTCAACCAATATTATATTACGCGTATAA^{m}CG^{m}CGTATTGCGCGCTATAATATTGAGGGAGAAGTGGTGA
Template-4m	CCTCACCATCTCAACCAATATTATATTA^{m}CG^{m}CGTATAA^{m}CG^{m}CGTATTGGCGCTATAATATTGAGGGAGAAGTGGTGA
Template-6m	CCTCACCATCTCAACCAATATTATATTA^{m}CG^{m}CGTATAA^{m}CG^{m}CGTATTG^{m}CG^{m}CGCTATAATATTGAGGGAGAAGTGGTGA

Name	Sequences (5'-3')
Forward primer-76mer templates	GGGTTTTATTATTTTAATTAATATTATATT
Reverse primer-76mer templates	TCACCACTTCTCCCTCAAT
Forward primer- E-cadherin	TAGTAATTTTAGGTTAGAGGGTTAT
Forward primer- E-cadherin	AAACTCACAAATACTTTACAATTCC

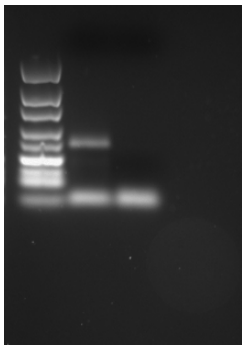

Figure **S1**. 2% gel electrophoresis results of asymmetry PCR without dGTP. Only DNA template without methylated cytosine can be amplified because no dGTP is needed to be incorporated in the complementary strand DNA (Lane 1, template-C). While methylcytosine remains intact and is still matched by Guanine in the complementary strand DNA after bisulfite treatment and PCR amplification, so no PCR products with expected length are observed (Lane 2, template-6m). This result is well consistent with the results of fluorescein-dGTP incorporated asymmetry PCR.

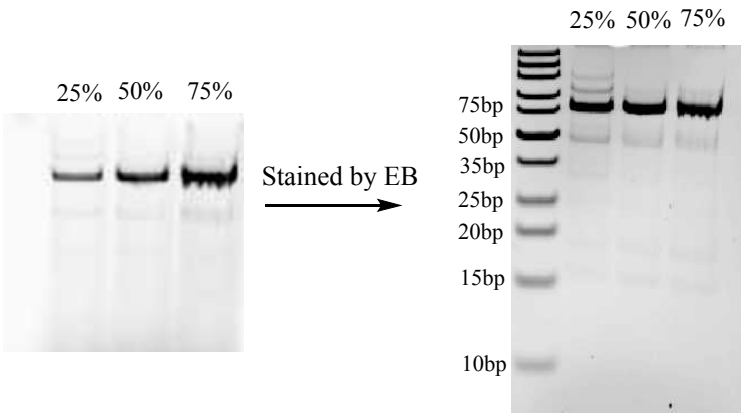

Figure **S2**. Different fractions of fluorescein-labeled dGTP in PCR amplification. When fluorescein-labeled dGTP is 75% of total dGTP, the corresponding fluorescence band of PCR product is the most intense and also exhibits efficient extension.