Supporting Information

FRET-Based Quantitative Discrimination of Bisulfite-Untreated DNA from Bisulfite-Treated DNA

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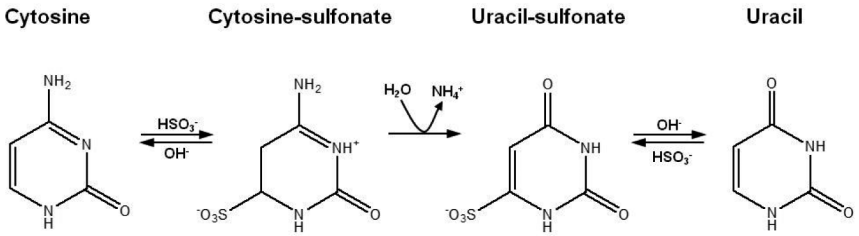

Figure S1. Bisulfite conversion (also known as bisulfite treatment) of DNA for methylation fine-mapping. Sodium bisulfite treatment of DNA results in conversion of unmethylated cytosines to uracil, leaving the methylated cytosines unconverted, and thus bisulfite treatment is considered the "gold standard" for downstream applications to assess DNA methylation status. Although this study presents experimental data for the primer-specific quantitation of DNA, our methodology for FRET measurement between QDs and intercalating dyes can be generally utilized to analyze DNA methylation, because bisulfite treatment is highly related with DNA methylation quantitation.

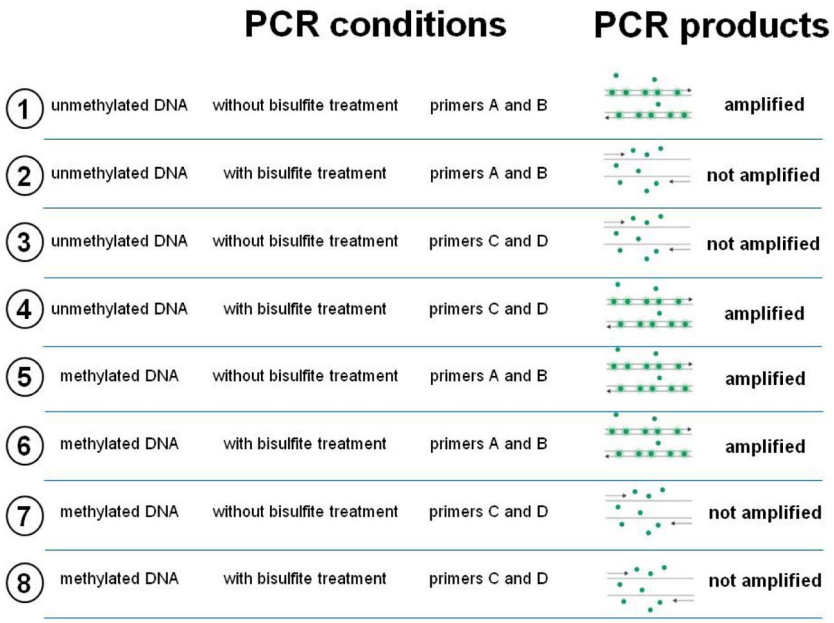

Figure S2. PCR conditions for combination of bisulfie treatment and primer sets in the presence of dsDNA-specific intercalating dyes. Primers A and B represent the forward and backward primers specific for bisulfite-untreated DNA templates, and Primers C and D stand for the forward and backward primers specific for bisulfite-treated DNA templates, respectively. Therefore, as illustrated, primers A and B work for PCR conditions of ①, ⑤, and ⑥, but not for ②, while primers C and D work for PCR conditions only of ④, but not for ③, ⑦, and ⑧.

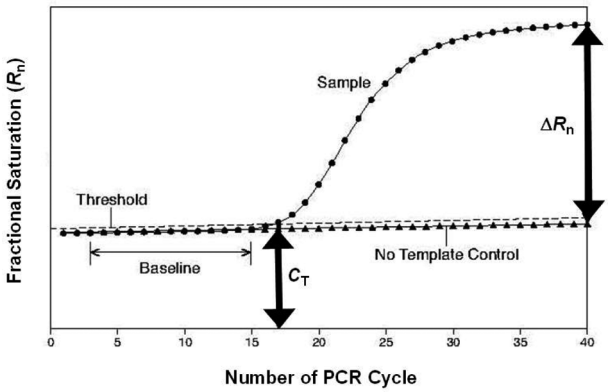

Figure S3. Principle of DNA quantitation based on C_T (cycle threshold) measurement. C_T is defined as the number of cycles for the fluorescent signal to cross the threshold (*i.e.* exceeds the background level). C_T levels are thus inversely proportional to the amount of target nucleic acid acid in the sample. R_n stands for the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye, and ΔR_n for R_n minus the baseline.

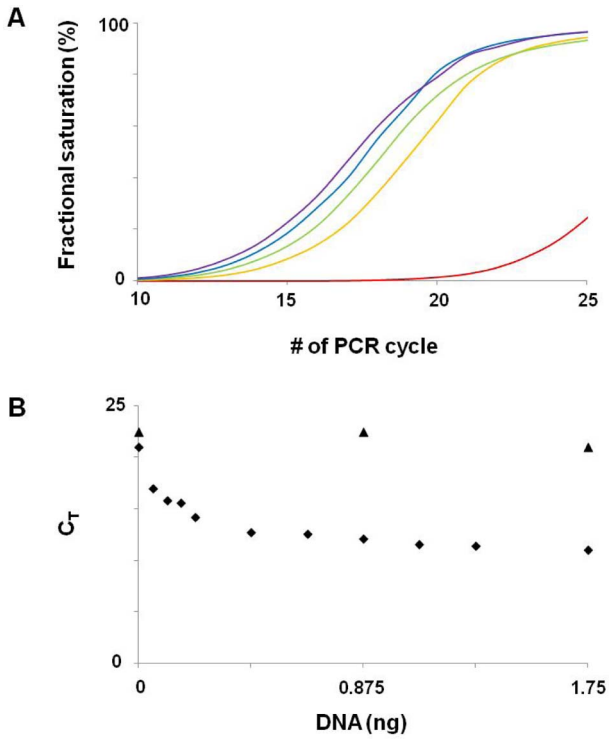

Figure S4. Measurement of C_T values of *hMLH1* gene promoter under the conditions of this study. A. Real-time PCR data of bisulfite-untreated DNA templates in the presence of primers A and B and SYBR Green, as the concentration of DNA templates is decreased (from left to right), resulting in measurement of C_T values of *hMLH1* gene promoter under the conditions of this study (quadrangle data in B). Triangle data represent C_T values of bisulfite-untreated DNA templates when using primers C and D instead of primers A and B. Similar data were obtained when bisulfite-treated DNA templates were amplified in the presence of primers C and D, and in the presence of primers A and B, respectively. It should be noted that C_T values against the initial amounts of DNA templates show a saturation curve, differently from the linear relationship in our system between the relative fluorescence intensities (F_{520}/F_{450}) and the the initial amounts of DNA templates (Figure 1B in the text).