

SUPPLEMENTARY MATERIAL FOR:

Reversal of PCR bias for improved sensitivity of the DNA methylation melting curve assay

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Bisulfite Conversion Protocol of Genomic DNA for Methylation Analyses^a

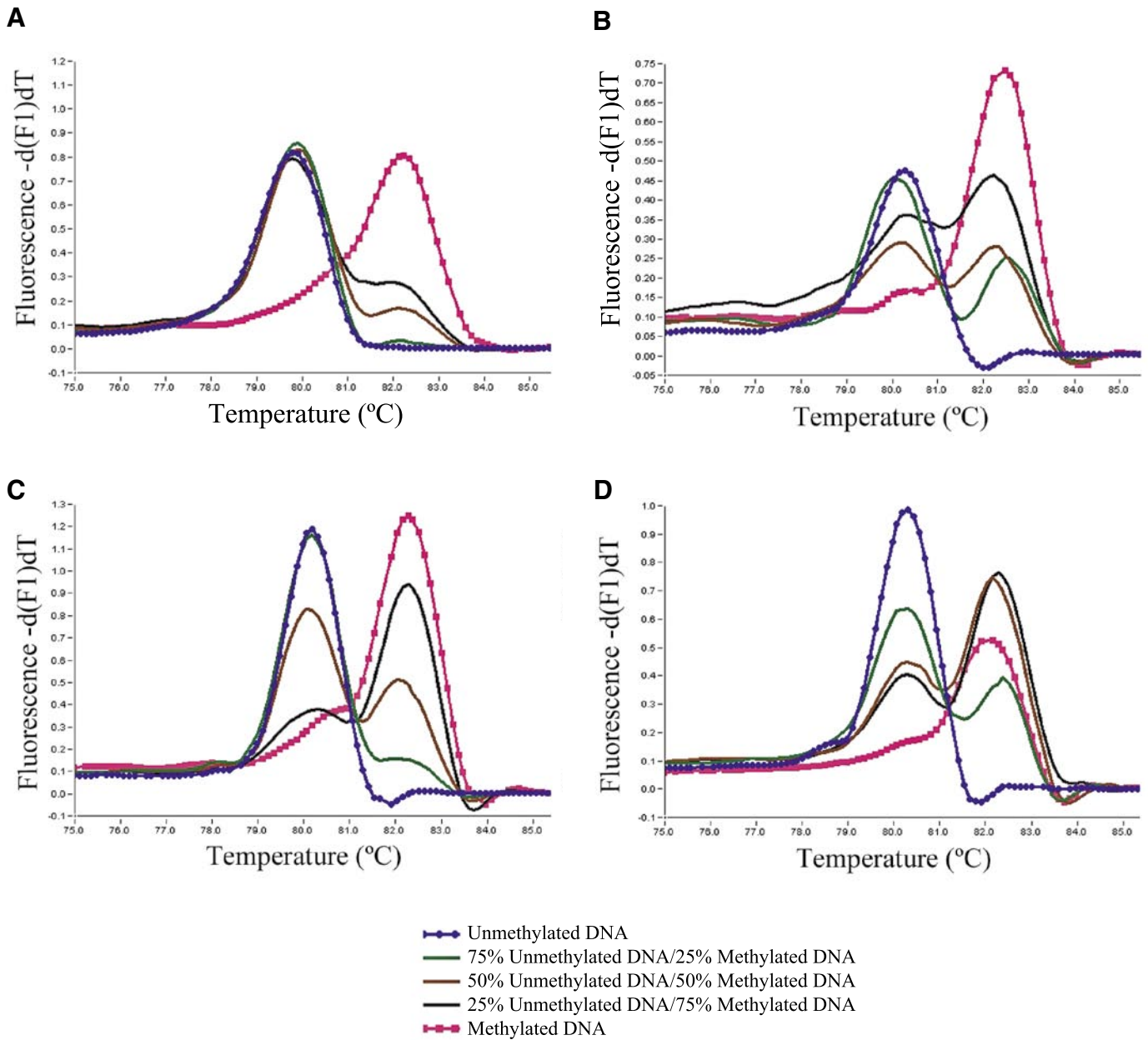
Reagents

1. 3 M sodium bisulfite
2. 10 mM hydroquinone
3. 3 M NaOH and 0.3 M NaOH

Procedure

1. Mix 200 ng DNA with water up to final volume 18 μ L.
2. Denature DNA by adding 2 μ L 3 M NaOH and incubating for 10 min at 37°C followed by 5 min at 95°C and directly place on ice.
3. Add 208 μ L freshly prepared 3 M sodium bisulfite (final concentration 2.6 M) and 12 μ L hydroquinone solution (final concentration 0.5 mM), mix with pipet, and incubate for 16 h (overnight) at 55°C.
4. Dilute the bisulfite reaction with water up to volume of 350–400 μ L.
5. Transfer this solution to an assembled Microcon YM-100 centrifugal filter unit.
6. Centrifuge at 2800 rpm using a Model 5417R centrifuge (Eppendorf, Westbury, NY, USA) for 10 min.
7. Discard the filtrate, add 250 μ L water to upper chamber, and centrifuge for 12 min at 2800 rpm.
8. Repeat step 7.
9. Discard the filtrate, add 300 μ L 0.3 M NaOH to the upper chamber, incubate at 37°C for 15 min, and centrifuge for 6 min at 2800 rpm.
10. Discard filtrate, add 250 μ L water to the upper chamber, and centrifuge at 2800 rpm for 15 min.
11. Elute the sample by adding 50 μ L Tris-EDTA (TE) buffer, use the pipet for mixing, and let stand for 15 min.
12. Invert the device and collect TE solution of the bisulfite-converted DNA in a clean tube.
13. Keep overnight at -20°C before use for analyses.

^aModified from Reference 9.



Supplementary Figure S1. Annealing temperature (T_a) dependent amplification of methylated and unmethylated variants of the sequence. Melting profiles of the PCR products amplified from the promoters of two putative tumor suppressor genes. Panels A and B sequence one amplified at T_a 61° or 68°C, respectively, and panels C and D sequence two amplified at T_a 60° and 64°C, respectively. The details regarding the identity of the putative tumor suppressor genes are in preparation for publication.