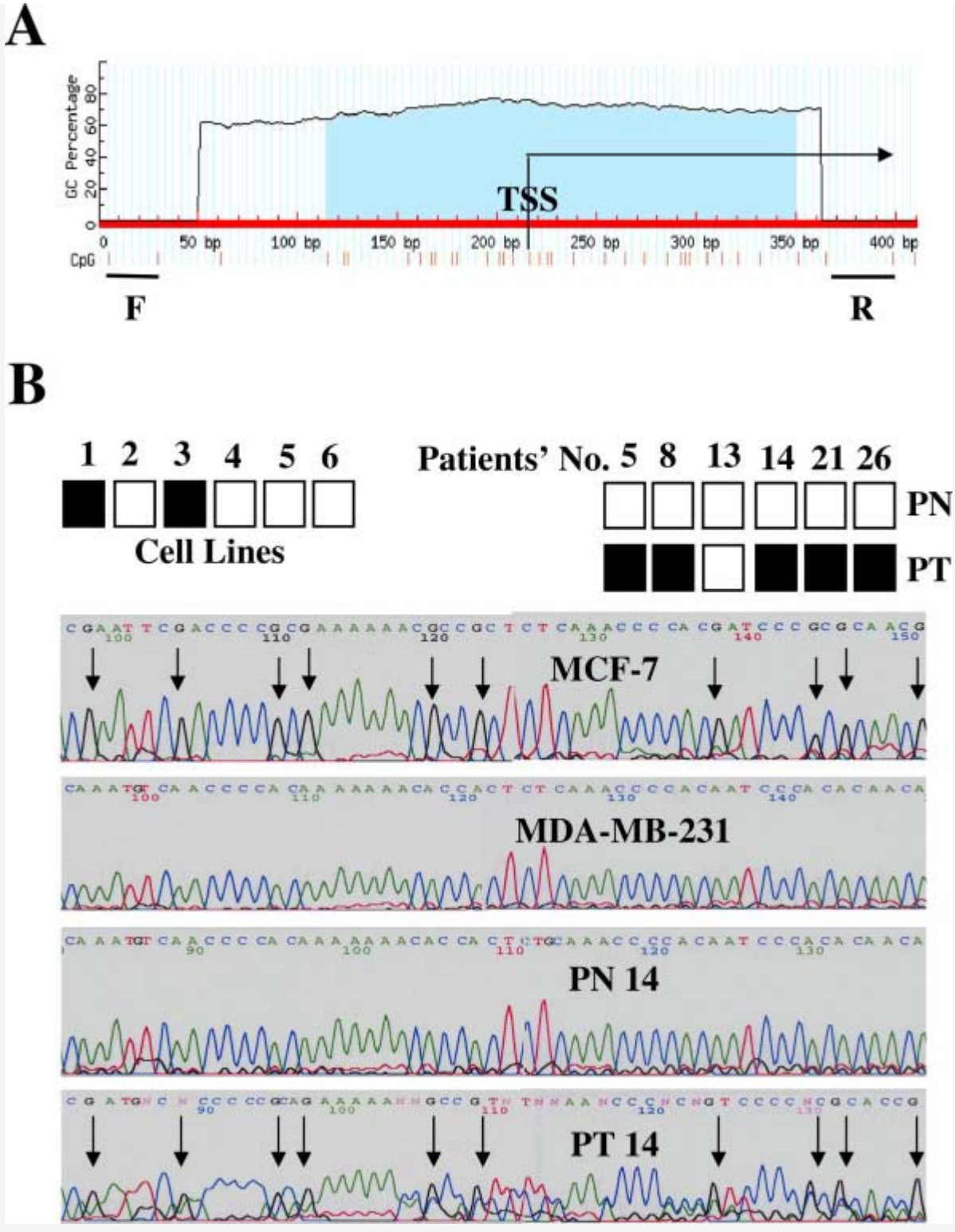
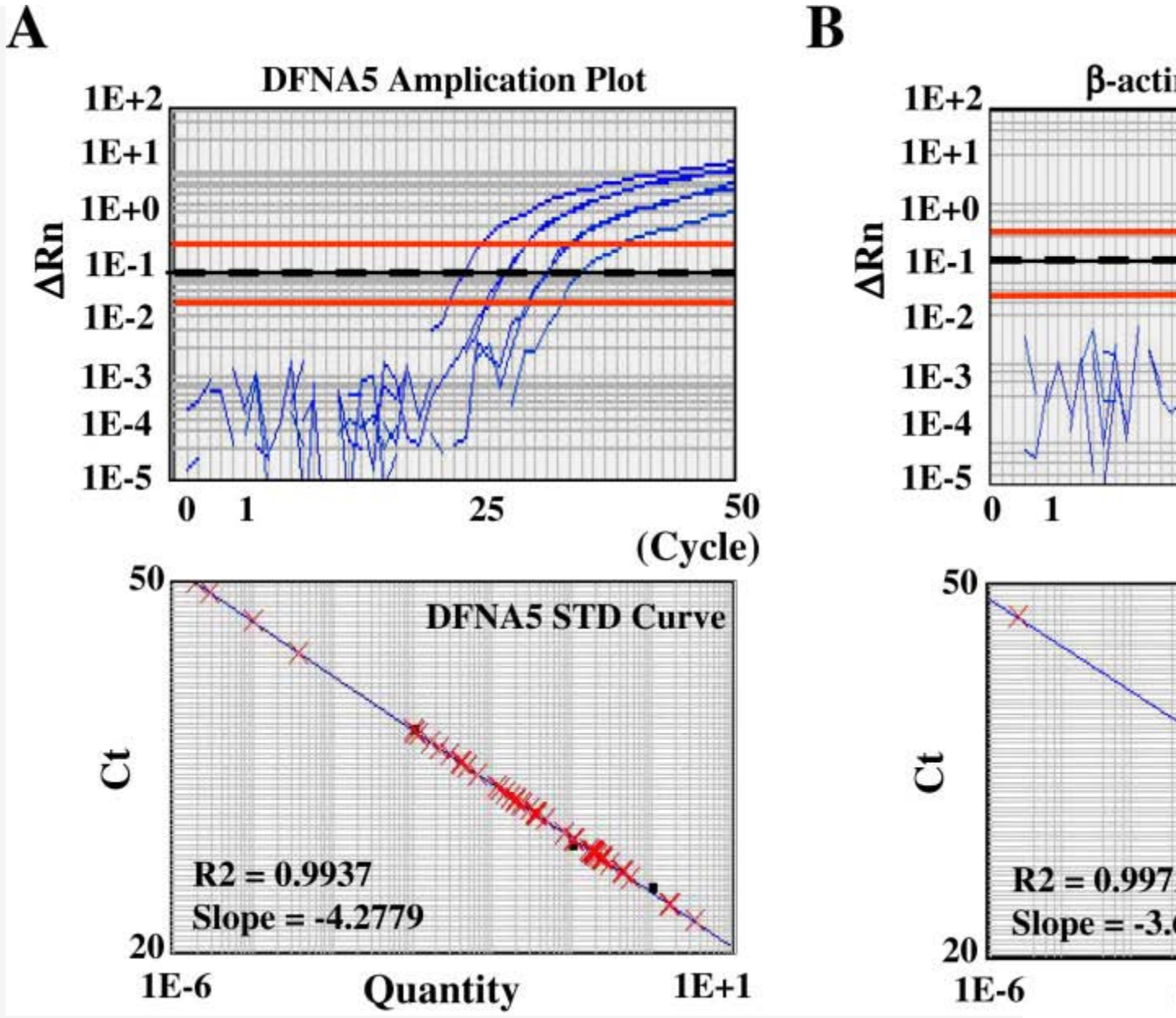
Appendix A. Supplementary data



Full-size image (129K)

Supplementary Fig. 1. Representative sequencing results of *DFNA5* gene promoter in breast cancer. (A) Graphics of CpG islands (gray color) are taken from Methprimer software. Bisulfite-sequencing primers were designed at the CpG

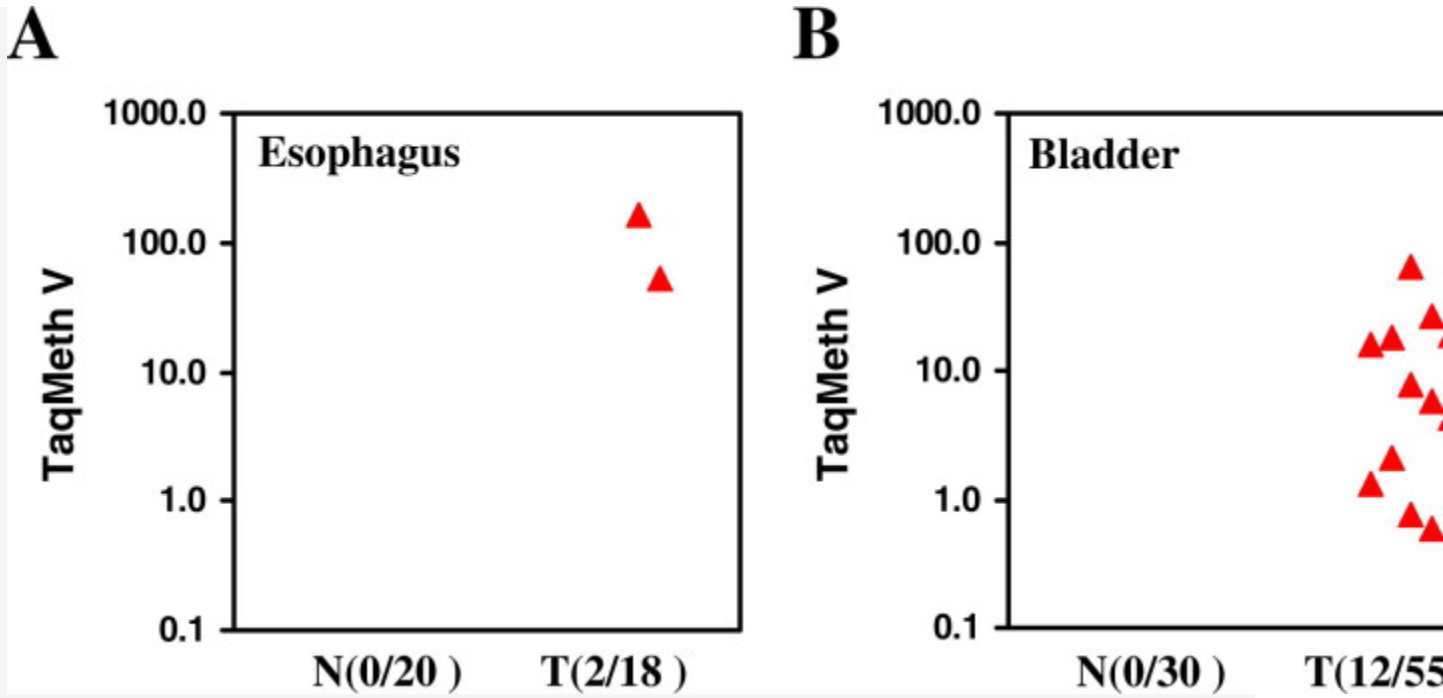
islands. F, forward; R, reverse primer. TSS, transcription start site. (B) Promoter methylation of the *DFNA5* was analyzed in MCF-12A and five breast cancer cell lines (MCF-7, BT-20, MDA-MB-231, Hs780, and Hs.578T) by bisulfite-sequencing. Methylation of *DFNA5* in MCF-7 and BT-20 was found but not in other cell lines. 1, MCF-7; 2, MDA-MB-231; 3, BT-20; 4, Hs780; 5, Hs.578T; 6, MCF-12A. We chose six pairs of normal and tumor samples to examine methylation status of the gene in primary tissue. Interestingly, no methylation of *DFNA5* was found in all matched normal breast tissues tested (0%) whereas methylation was clearly detected in 5 of 6 breast cancer tissues, suggesting tumor-specific promoter methylation. Representative sequencing results of *DFNA5* in breast cancer cell lines (MCF-7, MDA-MB-231) and primary tissues (patient's No. 14). When methylation is found in more than 50% of total CGs in amplified PCR products, it is considered as "methylation-positive". Black, methylation; white, no methylation. PT, paired breast cancer tissue from patients with breast cancer; PN, paired normal breast tissue. Arrows, all guanines present after sequencing that are complementary to methyl cytosines on the opposite DNA strand.



Full-size image (190K)

Supplementary Fig. 2. Standard plots and curves for *DFNA5* and β-actin amplification. Serially diluted, bisulfite-converted human lymphocyte DNA was used for generating standard plots and curves. Slopes of *DFNA5* and β-actin were −4.2779 and −3.635, respectively. A threshold (dash line) was chosen within the linear range of amplification of each standard indicated (solid lines). We did not observe any deletion of β-actin in all samples. One NN sample in which *DFNA5* methylation was undetectable (*) had a relatively low level of β-actin (C_t value, 38) because of its relatively low gDNA concentration after bisulfite treatment ($\leqslant$0.2 μg). The C_t value of β-actin amplification in 95% of samples ranged from 25 to 28 (Fig. 2F).

View Within Article



Full-size image (33K)

Supplementary Fig. 3. Methylation of *DFNA5* in primary esophageal and bladder cancers. *DFNA5* promoter methylation was investigated by real-time-MSP (TaqMan-MSP) analysis in 20 normal (N) and 18 tumor (T) tissues from esophageal cancer patients (A), and 30 normal and 55 tumor tissues from bladder cancer patients (B). *DFNA5* methylation was absent in all samples except two cases of ESCC (10%, 2/20) and 12 cases of bladder cancer (22%, 12/55). Samples with a ratio equal to zero could not be plotted correctly on a log scale, so are presented here as 0.001. TaqMeth V is described in Materials and methods.

View Within Article

☆Under a licensing agreement between OncoMethylome Sciences, SA and the Johns Hopkins University, Dr. Sidransky is entitled to a share of royalty received by the University on sales of products described in this article. Dr. Sidransky owns OncoMethylome Sciences, SA stock, which is subject to certain restrictions under University policy. Dr. Sidransky is a paid consultant to OncoMethylome Sciences, SA and is a paid member of the company's Scientific Advisory Board. The term of this arrangement is being managed by the Johns Hopkins University in accordance with its conflict of interest policies.

Corresponding author. Fax: +1 410 614 1411.