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TECHNICAL ADVANCE

Detection of Aberrant *TERT* Promoter Methylation by Combined Bisulfite Restriction Enzyme Analysis for Cancer Diagnosis

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Aberrant CpG dinucleotide methylation in a specific region of the telomerase reverse transcriptase (*TERT*) promoter is associated with increased TERT mRNA levels and malignancy in several cancer types. However, routine screening of this region to aid cancer diagnosis can be challenging because i) several established methylation assays may inaccurately report on hypermethylation of this particular region, ii) interpreting the results of methylation assays can sometimes be difficult for clinical laboratories, and iii) use of high-throughput methylation assays for a few patient samples can be cost prohibitive. Herein, we describe the use of combined bisulfite restriction enzyme analysis (COBRA) as a diagnostic tool for detecting the hypermethylated *TERT* promoter using *in vitro* methylated and unmethylated genomic DNA as well as genomic DNA from four melanomas and two benign melanocytic lesions. We compare COBRA with MassARRAY, a more commonly used high-throughput approach, in screening for promoter hypermethylation in 28 formalin-fixed, paraffin-embedded neuroblastoma samples. COBRA sensitively and specifically detected samples with hypermethylated *TERT* promoter and was as effective as MassARRAY at differentiating high-risk from benign or low-risk tumors. This study demonstrates the utility of this low-cost, technically straightforward, and easily interpretable assay for cancer diagnosis in tumors of an ambiguous nature. *(J Mol Diagn 2017, ■: 1–9; http://dx.doi.org/10.1016/j.jmoldx.2017.01.003)* Q5

Q6 The telomerase reverse transcriptase (*TERT*) oncogene encodes the rate-limiting catalytic subunit of telomerase, the enzyme required by virtually all proliferative cells to maintain the integrity of chromosomal ends.[1,2] Cancer cell lines and tissues have an uncontrolled capacity for proliferation, and TERT mRNA levels are inappropriately elevated through diverse mechanisms in approximately 85% to 90% of these cases.[3–5] Certain mechanisms of *TERT* dysregulation predominate in some cancer types but not others. For example, activating point mutations in the *TERT* promoter are common in cancers such as melanoma, glioblastoma, thyroid cancer, bladder cancer, and liver cancer,[6–12] but not in others such as bone and soft tissue sarcomas, gastrointestinal stromal tumors, gastric cancer, and pancreatic cancer.[13–16] Similarly, copy number amplification of *TERT* is more common in medulloblastoma, lung cancer, cervical cancer, and breast cancer,[17–20] whereas structural rearrangements involving *TERT* are found in B-cell malignancies, neuroblastoma (NBL), and chromophobe renal cell carcinoma.[10,21–23]

In addition to these genetic mechanisms of *TERT* dysregulation, a prominent epigenetic mechanism associated with cancer is CpG dinucleotide hypermethylation in the upstream of transcription start site (UTSS), a region located −541 to −483 bp upstream of the start codon of *TERT* (Figure 1A).[24,25] Hypermethylation of CpG [F1] dinucleotides in the UTSS is associated with increased TERT mRNA levels and poorer patient outcomes[24–26] and

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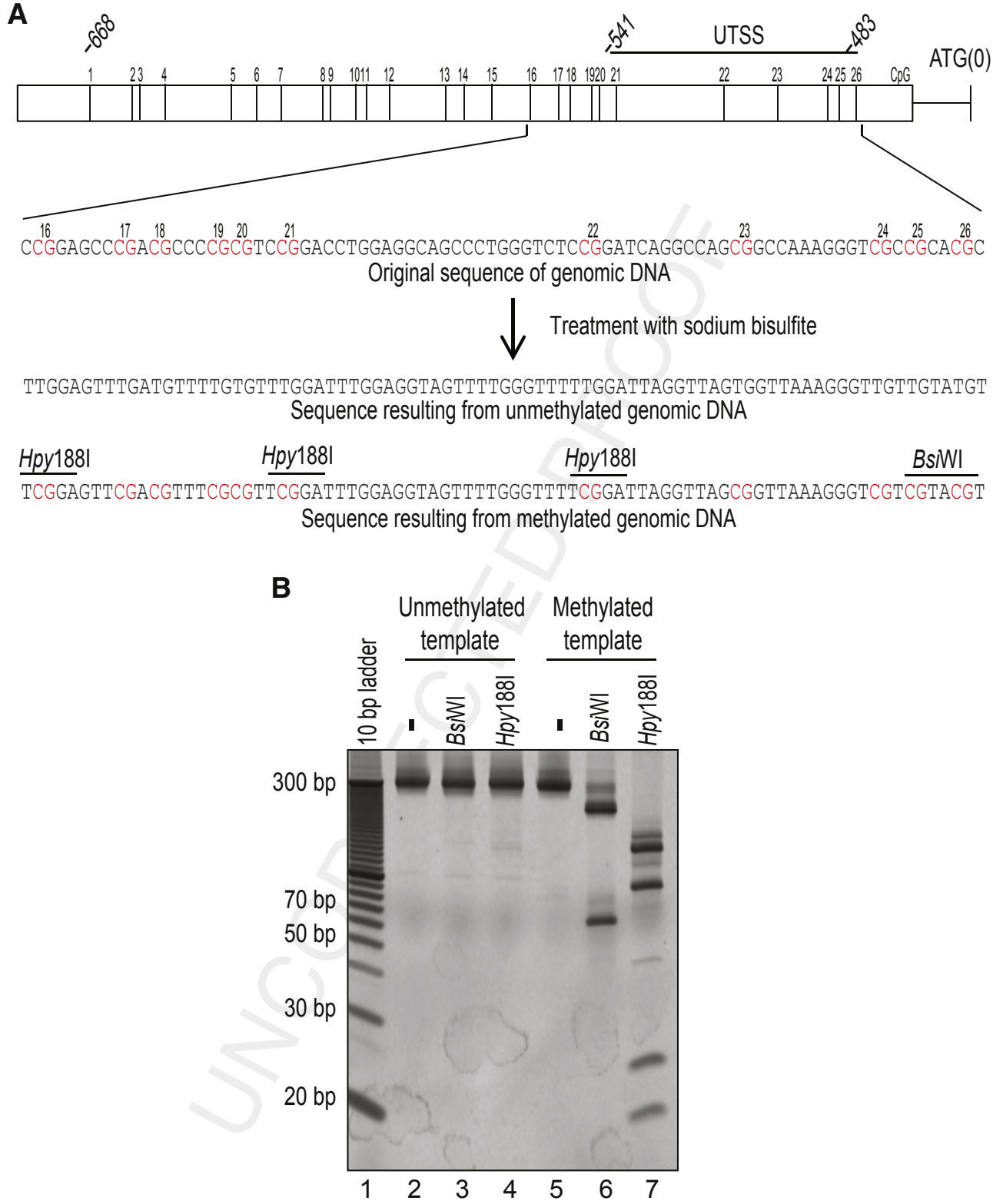

Figure 1 COBRA can distinguish hypermethylated from unmethylated UTSS through the PCR-RFLPs generated by the bisulfite treatment of genomic DNA. Q17 **A:** Map showing the positions of 26 CpG dinucleotides located in the proximal *TERT* promoter [nucleotides 1295772 to 1295104 on chromosome 5 (GRCh37/hg19)]. The six CpG dinucleotides within the UTSS, located −483 to −541 bp upstream of the translation start site, are indicated. The genomic sequence of the UTSS before bisulfite treatment, as well as the resulting sequences after bisulfite conversion if none or all of the CpG dinucleotides in the UTSS are protected by methylation, are shown. CpG dinucleotides are highlighted in red, and *Bsi*WI (5′-CGTACG-3′) and *Hpy*188I (5′-TCNGA-3′) restriction sites generated by bisulfite treatment are indicated. **B:** Restriction digestion of the UTSS PCR product from bisulfite-treated unmethylated (lanes 2 to 5) or methylated (lanes 5 to 7) genomic DNA. Identical Q18 Q19 blotches observed on a subset of gels are the result of residue present on the surface on which those gels were imaged.

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