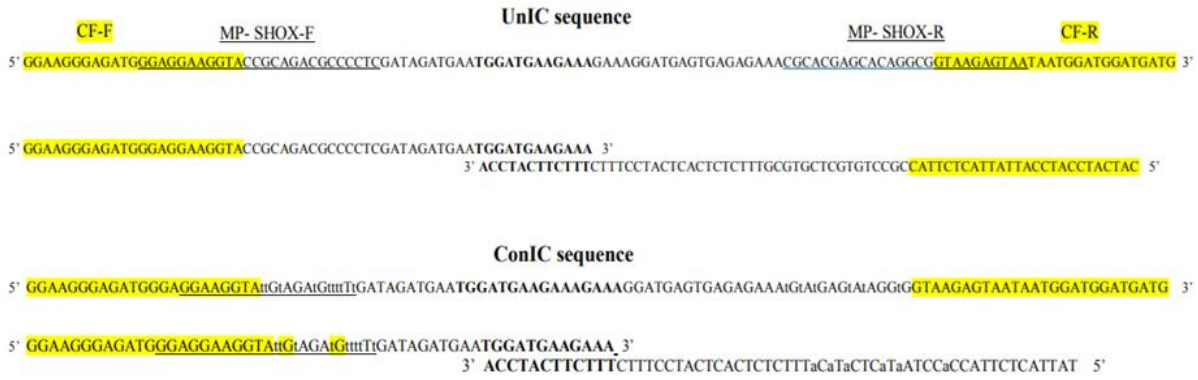
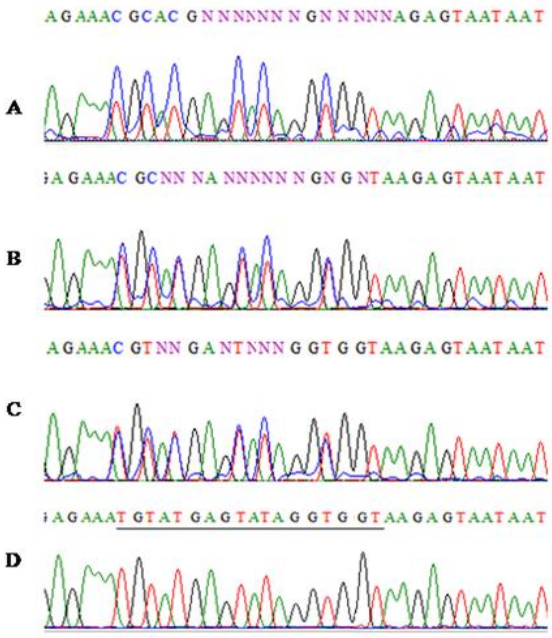
Supplementary Material

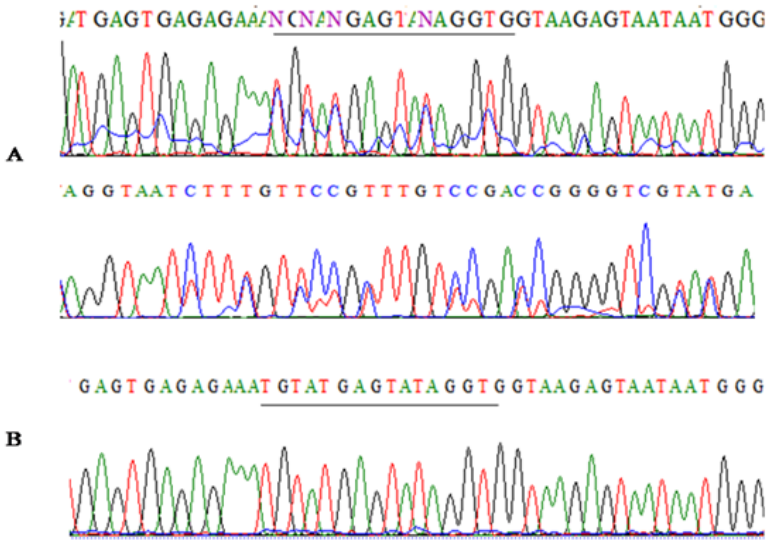
Lan et al. **An internal control for evaluating bisulfite conversion in the analysis of *Short Stature Homeobox 2* methylation in lung cancer**



Supplementary Figure 1. Nucleotide sequences (122 bp) of the UnIC and the ConIC fragments, and their synthesis by PCR extension. All "C" in the UnIC sequence were replaced by 't" in the ConIC sequence. The CF-F/R primer set (highlighted in yellow) targeting the CF sequence in the IC is used to evaluate DNA recovery, whereas the MP-SHOX2-F/R primer set (underlined) targeting the *SHOX2* sequence in IC is used to evaluate the bisulfite conversion efficiency.



Supplementary Figure 2. Direct sequencing of the UnIC insert. The UnIC insert was amplified from samples containing 5 ng (A), 0.5 ng (B), 0.05 ng (C) and 0.005 ng (D) of pUnIC that was mixed with 500 ng of genomic DNA and bisulfite converted. Cytosines that were not fully converted to thymines were remarked as N. The CpG containing *SHOX2* sequence inserted into the UnIC is underlined.



Supplementary Figure 3. Direct sequencing of the UnIC and *SHOX2* sequences. (A) Direct sequencing of the UnIC (upper) and *SHOX2* (lower) sequences amplified from sample containing 500 ng of genomic DNA plus 0.005 ng of pUnIC and insufficiently bisulfite converted as recommended by the manufacturer. The CpG containing *SHOX2* sequence inserted into the UnIC is underlined. (B) Direct sequencing of the UnIC sequences amplified from sample sufficiently bisulfite converted. Since *SHOX2* methylation level in this sample is only 0.03%, no PCR product is enough for sequencing.

Supplementary Table 1. Primer sets and quantitative real time PCR conditions for the evaluation of DNA recovery, bisulfite conversion and analysis of *SHOX2* methylation.

Primers	Sequence (5'–3')	Amplicon size (bp)	qPCR conditions
CF-F	GGAAGGGAGATGGGAGGAAGGTA	122	95°C 5 min, 40 cycles of (95°C 10 sec, 63°C 30 sec, 72°C 30 sec).
CF-R	CATTCTCATTATTACCTACCTACTAC		
MP-SHOX2-F	GGAGGAAGGTATTGTAGATGTTTTTT	94	
MP-SHOX2-R	TTACTCTTACCACCTATACTCATACA		
Me-SHOX2-F	AGTCGTATTCGTAGACGTTTTTC	127	95°C 5 min, 40 cycles of (94°C 30 sec, 63°C 30 sec, 72°C 30 sec).
Me-SHOX2-R	AAACGCCTATACTCGTACG		