

**Table S1.** ddPCR probes and primers for detecting TERT promoter mutations

Mutation type	Primer/probe	Sequence (5' to 3')	Fluorescent Dye and quencher	PCR Product Size (bp)
<i>TERT</i> C228T	fw_primer	CCCTCCCGGGTCC	-	64
	rev_primer	CCGCGGAAAGGAAGG	-	
	wt_probe	CGGAgGGGGCTGG	HEX_lowaBlack	
	mut_probe	CCCGGAaGGGGCTG	FAM_lowaBlack	
<i>TERT</i> C250T	fw_primer	TCCAGCTCCGCCTCCTCC	-	109
	rev_primer	GGGCCGCGGAAAGGAAGG	-	
	wt_probe	TCCCGACCCCTcCCGGGTCC	HEX_lowaBlack	
	mut_probe	TCCCGACCCCTtCCGGGTCC	FAM_lowaBlack	
<i>TERT</i> C228A	fw_primer	CGCGGAAAGGAAGGG	-	64
	rev_primer	CCCCTCCCGGGTC	-	
	wt_probe	CGGAgGGGGCTGG	HEX_lowaBlack	
	mut_probe	CCCGGAtGGGGCTG	FAM_lowaBlack	
<i>TERT</i> CC242-243TT	fw_primer	GAGGGCCCGGAGG	-	88
	rev_primer	CTTCACCTTCCAGCTCC	-	
	wt_probe	CTGGGCCGGgAC	HEX_lowaBlack	
	mut_probe	CCGGaaACCCGGGA	FAM_lowaBlack	
<i>TERT</i> A161C	fw_primer	CGGACCCCGCCCCGT	-	154
	rev_primer	CCAGGGCTTCCACGTGC	-	
	wt_probe	CAGCGCTGCCGGAAACTCG	HEX_lowaBlack	
	mut_probe	CAGCGCTGCCTGAAACTCGC	FAM_lowaBlack	

Fw: Forward; rev: Reverse; wt: Wild-type; mut: Mutated.

ddPCR probes containing either a 5'-FAM or 5'-HEX reporter dye and 3' Iowa Black® Fluorescent quencher were HPLC purified.

**Table S2.** Distribution of demographics and selected exposures among patients with primary and recurrent bladder cancer

<b>Characteristic</b>	<b>Primary BC (total n=11) N (%)</b>	<b>Recurrent BC (total n= 20) N (%)</b>	<b>P-value</b>
<b>Age (years) <sup>1</sup></b>	62.2 ± 7.22	66.05 ± 10.5	0.301
<b>Sex</b>			1.000
Male	9 (81%)	17 (85%)	
Female	2 (18%)	3 (15%)	
<b>Active smoking</b>	11 (100%)	15 (75%)	0.070
<b>Opium use</b>	10 (91%)	14 (70%)	0.183
<b>Hematuria</b>			0.173
Gross	9 (81%)	10 (50%)	
Microscopic	2 (18%)	7 (35%)	
<b>Tumor stage</b>			0.676
MIBC <sup>2</sup>	3 (27%)	4 (20%)	
NMIBC <sup>3</sup>	8 (73%)	16 (80%)	
<b>Tumor grade</b>			0.809
Low grade	6 (54%)	10 (50%)	
High grade	5 (45%)	10 (50%)	
<b>Urine cytology</b>			1.000
Positive	8 (73%)	13 (65%)	
Negative	3 (27%)	7 (35%)	

1: Age is illustrated as median ± standard deviation

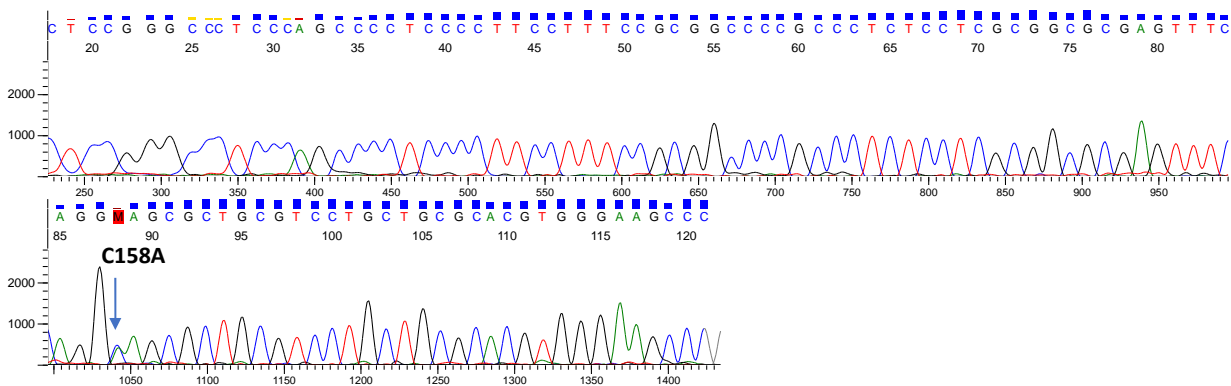
2. MIBC: Muscle-invasive bladder cancer

3. NMIBC: Non Muscle-invasive bladder cancer

**Table S3.** Distribution of the subtypes of TERT promoter mutations detected by ddPCR among participants in the case and control groups

Mutation type	Case group (total n=31) N (%)	Control group (total n= 50) N (%)	P-value
<i>TERT</i> C228T	18 (58%)	4 (8%)	<0.001
<i>TERT</i> C250T	4 (13%)	1 (2%)	0.068
<i>TERT</i> C228A	0	0	-
<i>TERT</i> CC242-243TT	0	0	-
<i>TERT</i> A161C	0	1 (2%)	0.617
Any of the above	20 (64.5%)	6 (12%)	<0.001

**Figure S1.** Sanger sequencing Chromatogram of the *TERT* promoter sequence of the KER-0023 urinary DNA sample identifying the C158A mutation.



The Mutant Allelic Fraction (MAF) of the C158A in the urinary DNA of this BC case is estimated to be at 50%.