

Figure S1. MB non-interference with RNA and cDNA using qPCR. Bar graph shows *18S rRNA* qPCR Ct values (Y-axis) from purified RNA incubated with (+) MB testing a series of dilutions (X-axis) and incubated with cDNA prior to qPCR testing a series of dilutions. RNA and cDNA (-) MB was used as a control.

Bioanalysis of RNA using isolation methods A - E and amplification efficiencies

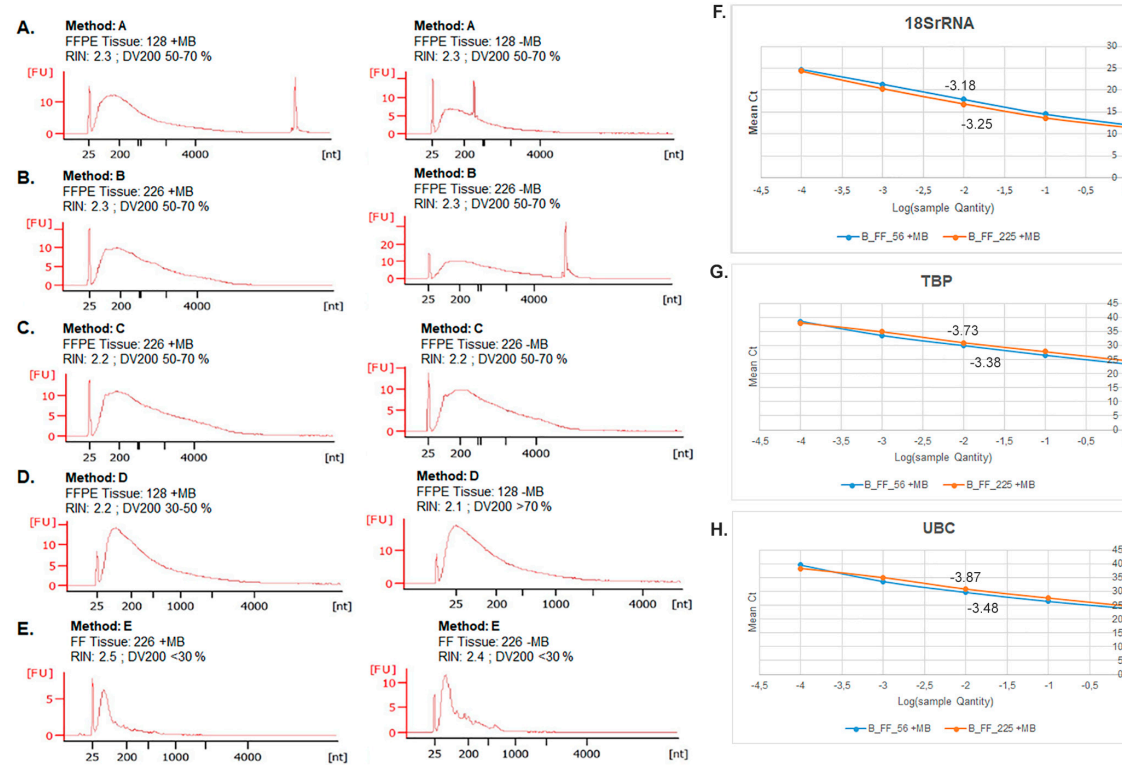


Figure S2. Bioanalysis of RNA from isolation methods and amplification efficiencies. A-E show chromatograms from RNA isolated from FFPE and FF tissues with Method A-E with or without MB, including the RIN and DV200 values for chosen examples; F-G: Two different MIBC RNAs were isolated using Method B with MB and were serially diluted to 1: 10.000. QPCR of *18S rRNA*, *TBP* and *UBC* was performed and standard curves created, which were used to calculate slopes (numbers above/below the curves).

Table S1. Top forward and bottom reverse primer for genes.

Gene name	TF (5'-3')	BR (5'-3')
<i>5SrRNA</i>	CCTGAACGCGCCCGATCT	TACAGCACCCGGTATTCCCA
<i>5.8SrRNA</i>	AGCGGTGGATCACTCGGC	CAAGTGC GTTCGAAGTGT CGA
<i>18SrRNA</i>	GCAATTATTCCCATGAACG	GGCCTCACTAAACCATCCAA
<i>GUSB</i>	ACGTGGTTGGAGAGCTCATTTG	GTTGTCTCTGCCGAGTGAAGA
<i>TBP</i>	GAAGTTGGGTTTTCCAGCTAAGT	ACTAAATTGTTGGTGGGTGAGC
<i>UBC</i>	GAAGATGGTCGTACCCTGTCT	GTCTTGCCAGTGAGTGTCTTC

Left to right columns: Gene name; top forward (TF) and bottom reverse (BR) primer nucleotide sequences for qPCR.

Table S2. Thirty-four reference genes and six MIBC subtyping target genes.

Gene name	Accession number	Gene Type
<i>ACTB</i>	NM_001101.5	Reference gene
<i>ALAS1</i>	NM_000688.6	Reference gene
<i>ATP5F1B</i>	NM_001686.4	Reference gene
<i>B2M</i>	NM_004048.4	Reference gene
<i>CFL1</i>	NM_005507.3	Reference gene
<i>CYC1</i>	NM_001916.5	Reference gene
<i>EIF4A2</i>	NM_001967.4	Reference gene
<i>GAPDH</i>	NM_002046.7	Reference gene
<i>GUSB</i>	NM_000181.4	Reference gene
<i>HMBS</i>	NM_000190.4	Reference gene
<i>HNRNPL</i>	NM_001533.3	Reference gene
<i>HPRT1</i>	NM_000194.3	Reference gene
<i>HSP90AB1</i>	NM_001371238.1	Reference gene
<i>PCBP1</i>	NM_006196.4	Reference gene

<i>PGK1</i>	NM_000291.4	Reference gene
<i>PPIA</i>	NM_021130.5	Reference gene
<i>RBCK1</i>	NM_006462.6	Reference gene
<i>RER1</i>	NM_007033.5	Reference gene
<i>RPL13</i>	NM_000977.4	Reference gene
<i>RPL29</i>	NM_000992.3	Reference gene
<i>RPL37A</i>	NM_000998.5	Reference gene
<i>RPLP0</i>	NM_001002.4	Reference gene
<i>RPS23</i>	NM_001025.5	Reference gene
<i>SDHA</i>	NM_004168.4	Reference gene
<i>TBP</i>	NM_003194.5	Reference gene
<i>TFRC</i>	NM_003234.4	Reference gene
<i>TSFM</i>	NM_001172696.2	Reference gene
<i>TMEM199</i>	NM_152464.3	Reference gene
<i>TUBB</i>	NM_001293212.2	Reference gene
<i>UBB</i>	NM_018955.4	Reference gene
<i>UBC</i>	NM_021009.7	Reference gene
<i>UBE2D2</i>	NM_003339.3	Reference gene
<i>WDR55</i>	NM_017706.5	Reference gene
<i>YWHAZ</i>	NM_145690.3	Reference gene
<i>KRT5</i>	NM_000424.4	Basal target gene
<i>KRT14</i>	NM_000526.5	Basal target gene
<i>CD44</i>	NM_000610.4	Basal target gene
<i>KRT20</i>	NM_019010.3	Luminal target gene
<i>GATA3</i>	NM_001002295.2	Luminal target gene
<i>FOXA1</i>	NM_004496.5	Luminal target gene

Table S3: Reference genes for BLCA / MIBC.

cancer	cohort [n] & tissues	controls [n] & tissues	RNA isolation	Analysis method	reference genes tested	best reference genes	literature reference
BLCA (transitional)	14 (FF)	matched	RNeasy Mini	qPCR	9	SDHA, TBP	53
BLCA (transitional)	35 (FF)	35 (matched)	Trizol	qPCR	18	RPS23, TPT1, RPS13	54
BLCA/ MIBC	28 (FFPE)	none	Roche FFPE	qPCR	20 (compared to TCGA)	UBC, RPL0, HMBS, GUSB, TBP	55
BLCA/ MIBC	29 (FFPE)	5 (bladder)	Promega-FFPE + MB	RNA hybrid.	34	UBC, GUSB, EIF4A2, WDR55, TBP	this study

Thirty-four reference genes and six MIBC subtyping target genes with NCBI Accession numbers (please see **Table S2**).