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Validation of an appropriate reference gene for normalization of reverse transcription-quantitative polymerase chain reaction data from rectal cancer biopsies.

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Supplementary Material

Supplementary Table 1

Genes examined

<i>Gene symbol</i>	<i>Gene name</i>	<i>GenBank accession no.</i>	<i>Function</i>	<i>Relative expression level</i>
Control gene (assessment of RT-qPCR inhibitors)				
<i>CAB</i>	<i>A. thaliana</i> chlorophyll a/b-binding protein	X56062	–	–
Candidate reference genes				
<i>18S</i>	18S ribosomal RNA	X03205	Part of a ribosomal subunit	Very high
<i>28S</i>	28S ribosomal RNA	M11167	Part of a ribosomal subunit	Very high
<i>ACTB</i>	Beta-actin	NM_001101	Cytoskeletal protein	High
<i>ATP6</i>	ATP synthase F0 subunit 6	NC_001807.4	Subunit of mitochondrial ATP synthase	High
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	NM_002046	Glycolysis enzyme	High
<i>ATUB</i>	Alpha tubulin	NM_006082	Component of microtubules	Medium
<i>B2M</i>	Beta-2-microglobulin	NM_004048	Beta chain of major histocompatibility complex antigen	Medium
<i>PGK1</i>	Phosphoglycerate kinase 1	NM_000291	Glycolysis enzyme	Medium
<i>RPLP0</i>	Ribosomal protein, large, P0	NM_001002	Component of the 60S subunit	Medium
<i>POLR2L</i>	Polymerase RNA II polypeptide L	NM_021128	Subunit of RNA polymerase II	Low
<i>PSMB6</i>	Proteasome subunit Y	NM_002798	Proteasome subunit	Low
<i>HMBS</i>	Hydroxymethylbilane synthase	NM_001024382	Enzyme involved in heme synthesis	Very low
<i>HPRT</i>	Hypoxanthine phosphoribosyl transferase 1	NM_000194	Central role in the generation of purine nucleotides	Very low
<i>PPIE</i>	Peptidylprolyl isomerase E	NM_203457	Involved in the folding of proteins	Very low
<i>TBP</i>	TATA box binding protein	NM_003194	Initiation of transcription by RNA polymerase II	Very low

Supplementary Table 2

Characteristics of the real-time PCR assays

Gene	Primer sequences (5' → 3')	Intron-spanning	Amplicon size (bp)	Annealing Temperature (°C)	qPCR efficiency (%)
<i>Control gene (assessment of RT-qPCR inhibitors)</i>					
<i>CAB</i>	F: CCATTGCATTTGTTGAGCAC R: CAATTCCTCGAGCTTCTTGG	Useless	119	62	100%
<i>Candidate reference genes</i>					
<i>18S</i>	F: GGCGCCCCCTCGATGCTCTTAG R: GCTCGGGCTGCTTTGAACACTCT	No	89	60	98%
<i>28S</i>	F: GGGGGAGAGGGTGTAATCT R: CAGAGGCTGTTACCTTGG	No	73	62	95%
<i>ACTB</i>	F: CTGTGGCATCCACGAAACTA R: AGTACTTGCGCTCAGGAGGA	Yes	200	60	100%
<i>ATP6</i>	F: AATGCCCTAGCCCCTTCTT R: CCAGGGCTATTGGTTGAATG	No	103	62	100%
<i>GAPDH</i>	F: TGCACCACCAACTGCTTAGC R: GGCATGGACTGTGGTCATGAG	Yes	87	64	100%
<i>ATUB</i>	F: TTACCTCGACTCTTAGCTTGTCTG R: GGATGGAGATGCACTCACG	Yes	107	60	92%
<i>B2M</i>	F: CACCCCACTGAAAAAGATG R: ATATTA AAAAAGCAAGCAAGCAGAA	Yes	167	60	93%
<i>PGK1</i>	F: GGGCTGCATCACCATCATA R: CCCCAGGAAGGACTTTACCTT	Yes	137	62	100%
<i>RPLP0</i>	F: CACTGAGATCAGGGACATGTTG R: CTCACATGGGGCAATGG	Yes	113	62	100%
<i>POLR2L</i>	F: CAACAAGTGGGAGGCTTACCT R: AGCTTCTCGATCAGGTCCAC	Yes	132	64	98%
<i>PSMB6</i>	F: GATACCGGAAGACCTGATG R: AATGGCAAAGGACTGCCTTA	Yes	116	60	99%
<i>HMBS</i>	F: TGTGGTGGGAACCAGCTC R: TGTGAGGTTTCCCCGAAT	Yes	92	64	100%
<i>HPRT</i>	F: TGATAGATCCATTCTATGACTGTAGA R: AAGACATTCTTTCCAGTTAAAGTTGAG	Yes	126	62	94%
<i>PPIE</i>	F: GCGTTCATTCTTTTGGAGA R: AAATCCTCGGTGCTTTCTG	Yes	75	60	99%
<i>TBP</i>	F: AAAGACCATTGCACTTCGTG R: GCACACCATTTTCCCAGAAC	Yes	124	52	100%

Forward and reverse primer sequences are indicated by “F” and “R”, respectively.