



HAL
open science

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

Alexandre Ho-Pun-Cheung, Caroline Bascoul-Mollevi, Eric Assénat, Frédéric Bibeau, Florence Boissière-Michot, Dominic Cellier, Marc Ychou, Evelyne Lopez-Crapez

► To cite this version:

Alexandre Ho-Pun-Cheung, Caroline Bascoul-Mollevi, Eric Assénat, Frédéric Bibeau, Florence Boissière-Michot, et al.. Validation of an appropriate reference gene for normalization of reverse transcription-quantitative polymerase chain reaction data from rectal cancer biopsies.: Normalization of RT-qPCR data in rectal cancer. *Analytical Biochemistry*, 2009, 388 (2), pp.348-50. 10.1016/j.ab.2009.03.001 . inserm-00396213

HAL Id: inserm-00396213

<https://inserm.hal.science/inserm-00396213>

Submitted on 19 Jun 2009

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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 (%)
Control gene (assessment of RT-qPCR inhibitors)					
<i>CAB</i>	F: CCATTGCATTTGTTGAGCAC R: CAATTCCTCGAGCTTCTTGG	Useless	119	62	100%
Candidate reference genes					
<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: AGTACTTGGCTCAGGAGGA	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 AAAAGCAAGCAAGCAGAA	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: TGTGAGGTTTCCCGAAT	Yes	92	64	100%
<i>HPRT</i>	F: TGATAGATCCATTCTATGACTGTAGA R: AAGACATTCTTCCAGTTAAAGTTGAG	Yes	126	62	94%
<i>PPIE</i>	F: GCGTTCATTCTTTTGGAGA R: AAATCCTCGGTGCTTTCTG	Yes	75	60	99%
<i>TBP</i>	F: AAAGACCATTGCACTTCGTG R: GCACACCATTTCCAGAAC	Yes	124	52	100%

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