

TABLE S2 MIQE Checklist			
ITEM TO CHECK	IMPORTANCE	CHECKLIST	METHOD DETAILS
EXPERIMENTAL DESIGN			
Definition of experimental and control groups	E	✓	
Number within each group	E	✓	>20
Assay carried out by core lab or investigator's lab?	D	✓	Investigator's lab
Acknowledgement of authors' contributions	D	✓	
SAMPLE			
Description	E	✓	
Volume/mass of sample processed	D		
Microdissection or macrodissection	E	Macro	
Processing procedure	E	✓	
If frozen - how and how quickly?	E	snap	
If fixed - with what, how quickly?	E	N/A	
Sample storage conditions and duration (especially for FFPE sample)	E	✓	Frozen in RNAlater
NUCLEIC ACID EXTRACTION			
Procedure and/or instrumentation	E	✓	
Name of kit and details of any modifications	E	✓	Qiagen RNeasy Plus Mini Kit #74134
Source of additional reagents used	D	✓	Worthington DNase I LS006344
Details of DNase or RNase treatment	E	✓	
Contamination assessment (DNA or RNA)	E	✓	primer sets that span exon/intron boundaries in RNA only qPCR reaction
Nucleic acid quantification	E	✓	
Instrument and method	E	✓	Nanodrop 2000C
Purity (A260/A280)	D	✓	
Yield	D	✓	
RNA integrity method/instrument	E		
RIN/RQI or Cq of 3' and 5' transcripts	E		
Electrophoresis traces	D		
Inhibition testing (Cq dilutions, spike or other)	E	✓	
REVERSE TRANSCRIPTION			
Complete reaction conditions	E	✓	65° 5 m, 42° 2 m, 42° 50 m, 70° 15 m, 37° 20 m
Amount of RNA and reaction volume	E	✓	1 ug RNA 20 ul reaction
Priming oligonucleotide (if using GSP) and concentration	E	✓	oligo d(T) 0.5 ug/ul stock
Reverse transcriptase and concentration	E	✓	Invitrogen SuperScript II # 100004925
Temperature and time	E	✓	
Manufacturer of reagents and catalogue numbers			NEB Rnase H # M0297L, Invitrogen RnaseOUT # 100000840, Invitrogen First Strand 5x buffer # Y02321
Cqs with and without RT	D	✓	
Storage conditions of cDNA	D	✓	negative 20 C
qPCR TARGET INFORMATION			
If multiplex, efficiency and LOD of each assay.	E	N/A	
Sequence accession number	E	✓	Table S2
Location of amplicon	D	✓	Table S3
Amplicon length	E	✓	Table S3
<i>In silico</i> specificity screen (BLAST, etc)	E	✓	PrimerBlast
Pseudogenes, retropseudogenes or other homologs?	D		
Sequence alignment	D	✓	PrimerBlast
Secondary structure analysis of amplicon	D	✓	PrimerBlast
Location of each primer by exon or intron (if applicable)	E	✓	
What splice variants are targeted?	E	✓	when possible all
qPCR OLIGONUCLEOTIDES			
Primer sequences	E	✓	Table S3
RTPrimerDB Identification Number	D	N/A	
Probe sequences	D	N/A	
Location and identity of any modifications	E	No mod	
Manufacturer of oligonucleotides	D	✓	Invitrogen
Purification method	D	✓	manufacturer's protocol
qPCR PROTOCOL			
Complete reaction conditions	E	✓	
Reaction volume and amount of cDNA/DNA	E	✓	25 ul 10 ng
Primer, (probe), Mg++ and dNTP concentrations	E	✓	600 nM
Polymerase identity and concentration	E	✓	Thermo Luminaris Color HiGreen Fluorescein qPCR Master Mix
Buffer/kit identity and manufacturer	E	✓	
Exact chemical constitution of the buffer	D	Manufacturer	
Additives (SYBR Green I, DMSO, etc.)	E	SYBR	
Manufacturer of plates/tubes and catalog number	D	✓	BioRad iCycler iQ PCR plates 2239441
Complete thermocycling parameters	E	✓	50° 2 m, 95° 10 m, 95° 15 s, 60° 1 m 40 cycles
Reaction setup (manual/robotic)	D	Manual	
Manufacturer of qPCR instrument	E	Biorad MyiQ	
qPCR VALIDATION			
Evidence of optimisation (from gradients)	D	✓	
Specificity (gel, sequence, melt, or digest)	E	Melt	
For SYBR Green I, Cq of the NTC	E	✓	
Standard curves with slope and y-intercept	E	N/A	
PCR efficiency calculated from slope	E	N/A	
Confidence interval for PCR efficiency or standard error	D	N/A	
r2 of standard curve	E	N/A	
Linear dynamic range	E	✓	
Cq variation at lower limit	E	✓	
Confidence intervals throughout range	D	✓	
Evidence for limit of detection	E	✓	
If multiplex, efficiency and LOD of each assay.	E	N/A	
DATA ANALYSIS			
qPCR analysis program (source, version)	E	✓	RefFinder, Prism 6
Cq method determination	E	✓	BioRad iCycler iQ
Outlier identification and disposition	E	No	
Results of NTCs	E	✓	
Justification of number and choice of reference genes	E	✓	RefFinder, Δ Cq, NormFinder, BestKeeper, geNorm
Description of normalisation method	E	✓	geNorm
Number and concordance of biological replicates	D		
Number and stage (RT or qPCR) of technical replicates	E	✓	2 replicates
Repeatability (intra-assay variation)	E	✓	
Reproducibility (inter-assay variation, %CV)	D	✓	
Power analysis	D		
Statistical methods for result significance	E	✓	one way ANOVA, student's t-test, linear regression
Software (source, version)	E	✓	Graphpad Prism 8
Cq or raw data submission using RDML	D	Raw only	