

Suppl. Table 1: Compliance of qPCR experiments with the MIQE guidelines

ITEM TO CHECK	IMPORTANCE	CHECKLIST
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	E	Yes (see manuscript for details)
Number within each group	E	Yes (see manuscript for details)
Assay carried out by core lab or investigator's lab?	D	Yes (see manuscript for details)
Acknowledgement of authors' contributions	D	No
SAMPLE		
Description	E	Yes (see manuscript for details)
Volume/mass of sample processed	D	Not applicable
Microdissection or macrodissection	E	Not applicable
Processing procedure	E	Not applicable
If frozen - how and how quickly?	E	Not applicable
If fixed - with what, how quickly?	E	Not applicable
Sample storage conditions and duration (especially for FFPE samples)	E	All RNA samples were stored at -80°C
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	E	Not applicable (Commercial)
Name of kit and details of any modifications	E	Yes (see manuscript for details)
Source of additional reagents used	D	Not applicable
Details of DNase or RNase treatment	E	Not applicable (Commercial)
Contamination assessment (DNA or RNA)	E	Yes (see manuscript for details)
Nucleic acid quantification	E	Yes (see manuscript for details)
Instrument and method	E	Yes (see manuscript for details)
Purity (A260/A280)	D	Yes (see manuscript for details)
Yield	D	Not applicable (Commercial)
RNA integrity method/instrument	E	Yes (see manuscript for details)
RIN/RQI or Cq of 3' and 5' transcripts	E	Not applicable (Commercial)
Electrophoresis traces	D	No
Inhibition testing (Cq dilutions, spike or other)	E	No (MIQE guidelines state that for screening studies these specifications are not required)
REVERSE TRANSCRIPTION		
Complete reaction conditions	E	Yes (see manuscript for details)
Amount of RNA and reaction volume	E	Yes (see manuscript for details)
Priming oligonucleotide (if using GSP) and concentration	E	No (Manufacturer does not provide stem-loop RT primer sequences)
Reverse transcriptase and concentration	E	Yes (see manuscript for details)
Temperature and time	E	Yes (see manuscript for details)
Manufacturer of reagents and catalogue numbers	D	Yes (see manuscript for details)
Cqs with and without RT	D*	No (see comment on DNase treatment)
Storage conditions of cDNA	D	cDNA was stored at -20°C
qPCR TARGET INFORMATION		
If multiplex, efficiency and LOD of each assay.	E	No (MIQE guidelines state that for screening studies these specifications are not required)
Sequence accession number	E	Yes (see manuscript for details)
Location of amplicon	D	No (primer sequence information given)
Amplicon length	E	Yes (see manuscript for details)
<i>In silico</i> specificity screen (BLAST, etc)	E	Yes (see manuscript for details)
Pseudogenes, retropseudogenes or other homologs?	D	No (MIQE guidelines state that for screening studies these specifications are not required)
Sequence alignment	D	No (MIQE guidelines state that for screening studies these specifications are not required)

Secondary structure analysis of amplicon	D	No (MIQE guidelines state that for screening studies these specifications are not required)
Location of each primer by exon or intron (if applicable)	E	Not applicable
What splice variants are targeted?	E	Not applicable

qPCR OLIGONUCLEOTIDES

Primer sequences	E	Yes (see manuscript for details)
RTPrimerDB Identification Number	D	Not available
Probe sequences	D**	Not applicable
Location and identity of any modifications	E	Not applicable
Manufacturer of oligonucleotides	D	INVITROGEN
Purification method	D	No

qPCR PROTOCOL

Complete reaction conditions	E	Yes (see manuscript for details)
Reaction volume and amount of cDNA/DNA	E	Yes (see manuscript for details)
Primer, (probe), Mg++ and dNTP concentrations	E	Yes (see manuscript for details)
Polymerase identity and concentration	E	Yes (see manuscript for details)
Buffer/kit identity and manufacturer	E	Yes (see manuscript for details)
Exact chemical constitution of the buffer	D	Yes (see manuscript for details)
Additives (SYBR Green I, DMSO, etc.)	E	Yes (see manuscript for details)
Manufacturer of plates/tubes and catalog number	D	Yes (see manuscript for details)
Complete thermocycling parameters	E	Yes (see manuscript for details)
Reaction setup (manual/robotic)	D	Yes (see manuscript for details)
Manufacturer of qPCR instrument	E	Yes (see manuscript for details)

qPCR VALIDATION

Evidence of optimisation (from gradients)	D	No (MIQE guidelines state that for screening studies these specifications are not required)
Specificity (gel, sequence, melt, or digest)	E	Yes (see manuscript for details)
For SYBR Green I, Cq of the NTC	E	Yes (see manuscript for details)
Standard curves with slope and y-intercept	E	Yes (see manuscript for details)
PCR efficiency calculated from slope	E	Yes (see manuscript for details)
Confidence interval for PCR efficiency or standard error	D	No (MIQE guidelines state that for screening studies these specifications are not required)
r2 of standard curve	E	Yes (see manuscript for details)
Linear dynamic range	E	Yes (see manuscript for details)
Cq variation at lower limit	E	No (MIQE guidelines state that for screening studies these specifications are not required)
Confidence intervals throughout range	D	No (MIQE guidelines state that for screening studies these specifications are not required)
Evidence for limit of detection	E	Yes (see manuscript for details)
If multiplex, efficiency and LOD of each assay.	E	Not applicable

DATA ANALYSIS

qPCR analysis program (source, version)	E	Yes (see manuscript for details)
Cq method determination	E	Yes (see manuscript for details)
Outlier identification and disposition	E	Yes (see manuscript for details)
Results of NTCs	E	Yes (see manuscript for details)
Justification of number and choice of reference genes	E	Yes (see manuscript for details)
Description of normalisation method	E	Yes (see manuscript for details)
Number and concordance of biological replicates	D	Yes (see manuscript for details)
Number and stage (RT or qPCR) of technical replicates	E	Yes (see manuscript for details)
Repeatability (intra-assay variation)	E	Yes (see manuscript for details)
Reproducibility (inter-assay variation, %CV)	D	Yes (see manuscript for details)
Power analysis	D	No
Statistical methods for result significance	E	No
Software (source, version)	E	No
Cq or raw data submission using RDML	D	No