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| **MIQE checklist and data set** |

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| **ITEM TO CHECK** | **IMPORTANCE** | **Comments** |
| **EXPERIMENTAL DESIGN** |  |  |
| Definition of experimental and control groups | **E** | Provided throughout manuscript |
| Number within each group | **E** | Provided throughout manuscript |
| Assay carried out by core lab or investigator's lab? | D | Yes. |
| Acknowledgement of authors' contributions | D | NA |
| **SAMPLE** |  |  |
| Description | **E** | Page 6 |
| Volume/mass of sample processed | D | Page 6 |
| Microdissection or macrodissection | **E** | NA |
| Processing procedure | **E** | Page 6 |
| If frozen - how and how quickly? | **E** | Page 6 |
| If fixed - with what, how quickly? | **E** | NA |
| Sample storage conditions and duration (especially for FFPE samples) | **E** | Page 6 |
| **NUCLEIC ACID EXTRACTION** |  |  |
| Procedure and/or instrumentation | **E** | Page 6 |
| Name of kit and details of any modifications | **E** | Page 6 |
| Source of additional reagents used | D | Page 6 |
| Details of DNase or RNAse treatment | **E** | Page 6 |
| Contamination assessment (DNA or RNA) | **E** | Page 6 and Supplemental Table 1 |
| Nucleic acid quantification | **E** | Page 6 |
| Instrument and method | **E** | Page 6 and Supplemental Table 1 |
| Purity (A260/A280) | D | Page 6 and Supplemental Table 1 |
| Yield | D | Page 6 and Supplemental Table 1 |
| RNA integrity method/instrument | **E** | Page 6 and Supplemental Table 1 |
| RIN/RQI or Cq of 3' and 5' transcripts | **E** | NA |
| Electrophoresis traces | D | NA |
| Inhibition testing (Cq dilutions, spike or other) | **E** | NA |
| **REVERSE TRANSCRIPTION** |  |  |
| Complete reaction conditions | **E** | Page 7 and Supplemental Tables 2 and 3 |
| Amount of RNA and reaction volume | **E** | Page 7 and Supplemental Tables 2 and 3 |
| Priming oligonucleotide (if using GSP) and concentration | **E** | NA |
| Reverse transcriptase and concentration | **E** | Page 7 and Supplemental Tables 2 and 3 |
| Temperature and time | **E** | Page 7 and Supplemental Tables 2 and 3 |
| Manufacturer of reagents and catalogue numbers | D | Page 7 |
| Cqs with and without RT | D\* | Throughout entire manuscript |
| Storage conditions of cDNA | D | -20C or Immediate Use |
| **qPCR TARGET INFORMATION** |  |  |
| If multiplex, efficiency and LOD of each assay. | **E** | Throughout manuscript. |
| Sequence accession number | **E** | NA |
| Location of amplicon | D | NA |
| Amplicon length | **E** | NA |
| In silico specificity screen (BLAST, etc) | **E** | NA |
| Pseudogenes, retropseudogenes or other homologs? | D | NA |
| Sequence alignment | D | NA |
| Secondary structure analysis of amplicon | D | NA |
| Location of each primer by exon or intron (if applicable) | **E** | NA |
| What splice variants are targeted? | **E** | NA |
| **qPCR OLIGONUCLEOTIDES** |  |  |
| Primer sequences | **E** | NA |
| RTPrimerDB Identification Number | D | NA |
| Probe sequences | D\*\* | NA |
| Location and identity of any modifications | **E** | NA |
| Manufacturer of oligonucleotides | D | NA |
| Purification method | D | NA |
| **qPCR PROTOCOL** |  |  |
| Complete reaction conditions | **E** | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Reaction volume and amount of cDNA/DNA | **E** | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Primer, (probe), Mg++ and dNTP concentrations | **E** | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Polymerase identity and concentration | **E** | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Buffer/kit identity and manufacturer | **E** | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Exact chemical constitution of the buffer | D | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Additives (SYBR Green I, DMSO, etc.) | **E** | NA |
| Manufacturer of plates/tubes and catalog number | D | ABI: Plates, Eppendorf: Tubes. |
| Complete thermocycling parameters | **E** | Pages 7,8 and 9. Supplemental Tables 4 and 5 |
| Reaction setup (manual/robotic) | D | Pages 7,8 and 9. Supplemental Tables 4 and 5 - Manual. |
| Manufacturer of qPCR instrument | **E** | Page 8. |
| **qPCR VALIDATION** |  |  |
| Evidence of optimisation (from gradients) | D | NA |
| Specificity (gel, sequence, melt, or digest) | **E** | NA |
| For SYBR Green I, Cq of the NTC | **E** | NA |
| Standard curves with slope and y-intercept | **E** | NA |
| PCR efficiency calculated from slope | **E** | Pages 7,8 and 9. Throughout Results Section (P11,12,13,14,15.) Supplemental Table 6. Tables 1 and 2 |
| Confidence interval for PCR efficiency or standard error | D | Throughout Results Section (P11,12,13,14,15.) Supplemental Table 6. Tables 1 and 2 |
| r2 of standard curve | **E** | NA |
| Linear dynamic range | **E** | NA |
| Cq variation at lower limit | **E** | Throughout Manuscript and Supplemental Table 6 |
| Confidence intervals throughout range | D | Throughout Manuscript and Supplemental Table 6 |
| Evidence for limit of detection | **E** | Yes. |
| If multiplex, efficiency and LOD of each assay. | **E** | Yes. |
| **DATA ANALYSIS** |  |  |
| qPCR analysis program (source, version) | **E** | Pages 7,8 and 9 |
| Cq method determination | **E** | Pages 7,8 and 9 |
| Outlier identification and disposition | **E** | Pages 7,8 and 9 |
| Results of NTCs | **E** | Throughout Manuscript. |
| Justification of number and choice of reference genes | **E** | Throughout Results Section (P11,12,13,14,15.) Supplemental Table 6 and Table 1 |
| Description of normalisation method | **E** | Yes when required. |
| Number and concordance of biological replicates | D | Pages 6,7,8, and 9 |
| Number and stage (RT or qPCR) of technical replicates | **E** | Pages 6,7,8, and 9 |
| Repeatability (intra-assay variation) | **E** | Yes. |
| Reproducibility (inter-assay variation, %CV) | D | NA |
| Power analysis | D | NA |
| Statistical methods for result significance | **E** | Throughout Manuscript. Table 1, Table 2. Pages 8 and 9 |
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| \* Assessing the absence of DNA using a no RT assay is essential when first extracting RNA. Once the sample has been validated as RDNA-free, inclusion of a no-RT control is desirable, but no longer essential. |  |  |
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| \*\* Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay |  |  |
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