**MIQE Checklist**

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| **ITEM TO CHECK** | **IMPORTANCE** | **CHECKLIST** |
| **EXPRIMNETAL DESIGN** |
| Definition of experiment and control group | E | Page 4, lines 95–104 |
| Number within each group | E | Page 4, lines 102–104 |
| Assay carried out by core lab or investigator's lab? | D | Page 7, lines 187–188 |
| Acknowledgement of author's contributions | D | Page 12, lines 378–384 |
| Description | E |  |
| Volume /mass of sammples processed | D | N/A |
| Microdissection or macrodissection | E | N/A |
| Processing procedure | E |  |
| If frozen-how and how quickly? | E | N/A |
| If fixed-with what how quickly? | E | N/A |
| Sample storage conditions and durarion especially for ffpe sample | E | N/A |
| **NUCLEIC ACID EXTRACTION** |
| Procedure and/or details of any modifications | E |  |
|  Name of kit and details of any modifications | E | Page 5, lines 141–149 |
|  Source of additional reagents used | D | Page 5, lines 141–149 |
| Details of DNase or RNase treatment | E | Page 5, lines 141–149 |
| Contamination assessment (DNA or RNA) | E | Page 5, lines 141–149 |
| Nucleic acid quantification | E |  |
|  Instrument and method | E | Page 5, lines 143–145 |
|  Purity(A260/A280) | D | Page 5, lines 143–145 |
|  Yield | D | N/A |
| RNA integrity method/instrument | E | Page 5, lines 143–145 |
|  RIN/RQI or Cq of 3’and 5'transcripts | E | Page 5, lines 143–145 |
|  Electrophoresis traces | D | N/A |
| Inhibition testing (Cq dilutions, spike or other) | E | N/A |
| **REVERSE TRANSCRIPTION** |
| Complete reaction conditions | E | Page 5, lines 146–148 |
|  Amount of RNA and reaction volume | E | Page 5, lines 146–148 |
|  Priming oligonucleotide and concentration | E | Table 1 |
|  Reverse transcriptase and concentrarion | E | Page 5, lines 146–155 |
|  Temperature and time | E | Page 5, lines 146–155 |
|  Manufacture of reagents and catalpgue numbers | D | Page 5, lines 146–155 |
| Cqs with and without RT | D | Page 5, lines 146–155 |
| Storage conditions od cDNA | D | Page 5, lines 146–155 |
| **qPCR TARGET INFORMATION** |
| If multiplex efficiency and LOD of each assay | E | N/A |
| Sequence accession number | E | N/A |
| Location of amplicion | D | N/A |
|  Amplicon length | E | N/A |
|  *In silico* specificity screen | E | N/A |
| Pseudogenes,retropsendogenes or other homologs | D |
|  Sequence alignmen | D |
|  Secondart structure analysis of amplicon | D | N/A |
| Location of each primier by exon or intron | E | N/A |
|  What splice variants are targeted | E | N/A |
| **qPCR OLIGONUCLEOTIDES** |
| Primer sequences | E | Table 2 |
| Rtprimer DB Identification number | D | N/A |
| Probe sequence | D | N/A |
| Location and identity of any modifications | E | N/A |
| Manofacture of oligonuclortides | D | Page 5, lines 146–149 |
| Purification method | D | N/A |
| **qPCR PROTOCOL** |
| Complete reaction conditions | E | Page 5, lines 140–143 |
|  Reaction volume and amount of cDNA/DNA | E | Page 5, lines 146 |
|  Primer,(probe),mg++,and dNTP concentrations | E | N/A |
|  Polymerase identity and concentration | E | Page 5, lines 140–145 |
|  Buffer/kit identity and manufacturer | E | Page 5, lines 146–149 |
|  Exact chemical constitution of the buffer | D | N/A |
|  Additives (SYBRGREENI,DMSO) | E | N/A |
| Manufacture of plates/tubes and catalog number | D | Page 5, lines 146 |
| Complete thermocycling parameters  | E | Page 5, lines 147–152 |
| Reaction setup (manual/robotic) | D | N/A |
| Manufacture of qpcr instrument | E | Page 5, lines 148–153 |
| **qPCR VALIDATION** |
| Evidence of optimasation(from gradients) | D | N/A |
| Specificity(gel,sequence,melt,or digest) | E | N/A |
| For SYBR green GREEN I,cq of the NTC | E | Page 5, lines 146–150 |
| Standard curves with slope and y-intercept | E | Page 5, lines 146–150 |
|  PCR effiency calculated from slope | E | N/A |
|  Confidence interval for pcr efficiency or standard error | D | Page 5, lines 146–150 |
|  r2 of standard curve | E | Page 5, lines 146–150 |
| Linear dynamic range  | E | N/A |
|  Cq variation at lower limit | E | N/A |
| Confidence intervals throughout range | D | N/A |
| Evidence for limit of detection | E | N/A |
| If multiplex efficiency and LOD of each assay | E | N/A |
| **DATA ANALYSIS** |
| qPCR analysis program (source,version) | E | N/A |
| Cq method determination | E | Page 5, lines 153 |
| Outlier identification and disposition | E | N/A |
| Result of NTCs | E | N/A |
| Justification of number and choice of reference genes | E | Page 5, lines 154 |
| Description of normalisation method | E | Page 5, lines 154-155 |
| Number and concordance of biological replicates | D | Page 5, lines 154 |
| Number and stage (RT or qpcr)of technical replicates | E | N/A |
| Repeatability (intra assay variation,) | E | N/A |
| Repeatability (intra assay variation,%CV) | D | N/A |
| Power analysis | D | N/A |
| Statistical methods for result significance | E | Page 7, lines 187-191 |
| Software(source,version) | E | Page 5, lines 149-151 |
| Cq or raw data submission using RDML | D | N/A |