**MIQE Checklist**

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| **ITEM TO CHECK** | **IMPORTANCE** | **CHECKLIST** |
| **EXPRIMNETAL DESIGN** | | |
| Definition of experiment and control group | E | Cell culture and transfection section/line 70-78 |
| Number within each group | E | Cell culture and transfection section, line 79 |
| Assay carried out by core lab or investigator's lab? | D | Cell culture and transfection section, line 78 |
| Acknowledgement of author's contributions | D | None |
| Description | E |  |
| Volume /mass of sammples processed | D | qRT-PCR section, line 81 |
| Microdissection or macrodissection | E | None |
| Processing procedure | E |  |
| If frozen-how and how quickly? | E | None |
| If fixed-with what how quickly? | E | None |
| Sample storage conditions and durarion especially for ffpe sample | E | None |
| **NUCLEIC ACID EXTRACTION** | | |
| Procedure and/or details of any modifications | E |  |
| Name of kit and details of any modifications | E | RT-qPCR assay section/line 81-94 |
| Source of additional reagents used | D | RT-qPCR assay section/line 81-94 |
| Details of DNase or RNase treatment | E | RT-qPCR assay section/line 81-94 |
| Contamination assessment (DNA or RNA) | E | RT-qPCR assay section/line 81-94 |
| Nucleic acid quantification | E |  |
| Instrument and method | E | RT-qPCR assay section/line 81-94 |
| Purity(A260/A280) | D | RT-qPCR assay section/line 81-94 |
| Yield | D | RT-qPCR assay section/line 81-94 |
| RNA integrity method/instrument | E |  |
| RIN/RQI or Cq of 3’and 5'transcripts | E | RT-qPCR assay section/line 81-94 |
| Electrophoresis traces | D | RT-qPCR assay section/line 81-94 |
| Inhibition testing (Cq dilutions, spike or other) | E | RT-qPCR assay section/line 81-94 |
| **REVERSE TRANSCRIPTION** | | |
| Complete reaction conditions | E | RT-qPCR assay section/line 81-94 |
| Amount of RNA and reaction volume | E | RT-qPCR assay section/line 81-94 |
| Priming oligonucleotide and concentration | E | RT-qPCR assay section/line 81-94 |
| Reverse transcriptase and concentration | E | RT-qPCR assay section/line 81-94 |
| Temperature and time | E | RT-qPCR assay section/line 81-94 |
| Manufacture of reagents and catalogue numbers | D | None |
| Cqs with and without RT | D | None |
| Storage conditions od cDNA | D | None |
| **qPCR TARGET INFORMATION** | | |
| If multiplex efficiency and LOD of each assay | E | Yes |
| Sequence accession number | E | NR\_002819.4 |
| Location of amplicion | D | RT-qPCR assay section/line 81-94 |
| Amplicon length | E | RT-qPCR assay section/line 81-94 |
| *In silico* specificity screen | E | RT-qPCR assay section/line 81-94 |
| Pseudogenes,retropsendogenes or other homologs | D |
| Sequence alignmen | D |
| Secondart structure analysis of amplicon | D | RT-qPCR assay section/line 81-94 |
| Location of each primier by exon or intron | E | RT-qPCR assay section/line 81-94 |
| What splice variants are targeted | E | RT-qPCR assay section/line 81-94 |
| **qPCR OLIGONUCLEOTIDES** | | |
| Primer sequences | E | RT-qPCR assay section/line 81-94 |
| Rtprimer DB Identification number | D | None |
| Probe sequence | D | None |
| Location and identity of any modifications | E | None |
| Manofacture of oligonuclortides | D | None |
| Purification method | D | None |
| **qPCR PROTOCOL** | | |
| Complete reaction conditions | E | RT-qPCR assay section/line 81-94 |
| Reaction volume and amount of cDNA/DNA | E | RT-qPCR assay section/line 81-94 |
| Primer,(probe),mg++,and dNTP concentrations | E | RT-qPCR assay section/line 81-94 |
| Polymerase identity and concentration | E | RT-qPCR assay section/line 81-94 |
| Buffer/kit identity and manufacturer | E | RT-qPCR assay section/line 81-94 |
| Exact chemical constitution of the buffer | D |  |
| Additives (SYBRGREENI,DMSO) | E | None |
| Manufacture of plates/tubes and catalog number | D | RT-qPCR assay section/line 81-94 |
| Complete thermocycling parameters | E | RT-qPCR assay section/line 81-94 |
| Reaction setup (manual/robotic) | D | RT-qPCR assay section/line 81-94 |
| Manufacture of qpcr instrument | E | RT-qPCR assay section/line 81-94 |
| **qPCR VALIDATION** | | |
| Evidence of optimasation(from gradients) | D | RT-qPCR assay section/line 81-94 |
| Specificity(gel,sequence,melt,or digest) | E | RT-qPCR assay section/line 81-94 |
| For SYBR green GREEN I,cq of the NTC | E | None |
| Standard curves with slope and y-intercept | E | RT-qPCR assay section/line 81-94 |
| PCR effiency calculated from slope | E | RT-qPCR assay section/line 81-94 |
| Confidence interval for pcr efficiency or standard error | D | None |
| r2 of standard curve | E | RT-qPCR assay section/line 81-94 |
| Linear dynamic range | E | None |
| Cq variation at lower limit | E | None |
| Confidence intervals throughout range | D | None |
| Evidence for limit of detection | E | None |
| If multiplex efficiency and LOD of each assay | E | None |
| **DATA ANALYSIS** | | |
| qPCR analysis program (source,version) | E | RT-qPCR assay section/line 81-94 |
| Cq method determination | E | Quantitative reverse transcription-polymerase chain |
| Outlier identification and disposition | E | None |
| Result of NTCs | E | None |
| Justification of number and choice of reference genes | E | None |
| Description of normalisation method | E | Quantitative reverse transcription-polymerase chain |
| Number and concordance of biological replicates | D | None |
| Number and stage (RT or qpcr)of technical replicates | E | Quantitative reverse transcription-polymerase chain |
| Repeatability (intra assay variation,) | E | None |
| Repeatability (intra assay variation,%CV) | D | None |
| Power analysis | D | None |
| Statistical methods for result significance | E | Statistical analysis section/line 120-123. |
| Software(source,version) | E | Statistical analysis section/line 120-123. |
| Cq or raw data submission using RDML | D | None |