**Essential MIQE Checklist**

|  |  |  |
| --- | --- | --- |
| **Experimental Design** | |  |
| Definition of experimental and control groups | page5:156-157 | ∨ |
| Number within each group | page5:156-157, page11:350-360 | ∨ |
| **Sample** | |  |
| Description | page4:120-131 | ∨ |
| Microdissection or macrodissection | Not Applicable | ∨ |
| Processing procedure | page4:130-131 | ∨ |
| If frozen - how and how quickly? | page4:130-131 | ∨ |
| If fixed - with what, how quickly? | Not Applicable | ∨ |
| Sample storage conditions and duration (especially for FFPE samples) | page4:130-131 | ∨ |
| **Nucleic Acid Extraction** | |  |
| Procedure and/or instrumentation | page5-6:155-193 | ∨ |
| Name of kit and details of any modifications | page5-6:155-193 | ∨ |
| Details of DNase or RNAse treatment | page5:159-160 | ∨ |
| Contamination assessment (DNA or RNA) | page5:159-163 | ∨ |
| Nucleic acid quantification | page5:159-163 | ∨ |
| Instrument and method | page5:159-163 | ∨ |
| RNA integrity method/instrument | page5:159-163, and Table 1 | ∨ |
| RIN/RQI or Cq of 3' and 5' transcripts | page5:159-163, and Table 1 | ∨ |
| Table 1. RNA quality information   |  |  |  |  |  |  | | --- | --- | --- | --- | --- | --- | | Sample Name | Conc.(ng/ μL) | Final Volume (μL) | Total Amount (μg) | RIN | rRNA Ration | | PL1\_0h | 1618.106 | 16 | 25.89 | 9.4 | 1.6 | | PL1\_3h | 1511.103 | 22 | 33.244 | 9 | 1.6 | | PL1\_24h | 1577.114 | 20 | 31.4542 | 9.2 | 1.5 | | PL1\_48h | 1352.761 | 18 | 24.35 | 9.2 | 1.7 | | PL6\_0h | 2629.068 | 15 | 39.436 | 9.3 | 1.7 | | PL6\_3h | 1138.332 | 20 | 22.767 | 8.7 | 1.8 | | PL6\_24h | 1452.326 | 16 | 23.237 | 9.2 | 1.8 | | PL6\_48h | 2367.427 | 19 | 44.981 | 9.1 | 1.8 | | |  |
| Inhibition testing (Cq dilutions, spike or other) | Not Applicable | ∨ |
| **Reverse Transcription** | |  |
| Complete reaction conditions | page6:186-193 | ∨ |
| Amount of RNA and reaction volume | page6:186-187 | ∨ |
| Priming oligonucleotide (if using GSP) and concentration | Oligo dT, page6:186-187 | ∨ |
| Reverse transcriptase and concentration | page6:186-187 | ∨ |
| Temperature and time: page6:186-187 | page6:186-187 | ∨ |
| **qPCR Target** | |  |
| Gene symbol | page10:350-360 | ∨ |
| Sequence accession number | *TRAESCS1B02G095800, TRAESCS1B02G038700, TRAESCS1B02G102200, TRAESCS1B02G138100, TRAESCS2A02G046200, TRAESCS1B02G048900, TRAESCS1B02G071800, TRAESCS7D02G246600, TRAESCS6B02G466700, TRAESCS6B02G017900, TRAESCS5D02G537600, TRAESCS1B02G105100* |  |
| Amplicon length | Table 2 | ∨ |
| *In silico* specificity screen (BLAST, etc) | Table 2 | ∨ |
| Table 2. Amplicon length of RT-qPCR products   |  |  |  |  | | --- | --- | --- | --- | | Group | Accession Number | Blast description | Amplicon length (bp) | | Cluster 1 | *TRAESCS1B02G095800* | peroxidase 2 | 144 | | *TRAESCS1B02G038700* | protein NRT1/ PTR FAMILY 6.2 | 70 | | *TRAESCS1B02G138100* | auxin-responsive protein IAA15 | 159 | | *TRAESCS1B02G102200* | replication protein A 70 kDa DNA-binding subunit C-like | 143 | | Cluster 2 | *TRAESCS2A02G046200* | nuclear transport factor 2 (NTF2)-like protein | 82 | | *TRAESCS1B02G048900* | histone H2A | 162 | | *TRAESCS1B02G071800* | thylakoid membrane protein TERC, chloroplastic | 99 | | *TRAESCS7D02G246600* | probable histone H2A variant 3 | 181 | | Cluster 3 | *TRAESCS6B02G466700* | protein argonaute 1C-like isoform X2 | 121 | | *TRAESCS1B02G105100* | ribosome biogenesis protein NOP53 | 168 | | *TRAESCS6B02G017900* | predicted protein | 165 | | *TRAESCS5D02G537600* | aspartokinase 1, chloroplastic-like | 105 | | |  |
| Location of each primer by exon or intron (if applicable) | exon | ∨ |
| What splice variants are targeted? | Not Applicable | ∨ |
| **qPCR Oligos** | |  |
| Primer sequences | Refer to Supplementary Table 1 (Table S1) | ∨ |
| Location and identity of any modifications | Not Applicable | ∨ |
| **qPCR Protocol** | |  |
| Reaction volume and amount of cDNA/DNA | Page6:188-193 | ∨ |
| Primer, (probe), Mg++ and dNTP concentrations | Page6:188-193 | ∨ |
| Polymerase identity and concentration | Page6:188-193 | ∨ |
| Buffer/kit identity and manufacturer | Page6:188-193 | ∨ |
| Additives (SYBR Green I, DMSO, etc.) | Page6:188-193 | ∨ |
| Complete thermocycling parameters | Page6:188-193 | ∨ |
| Manufacturer of qPCR instrument | Page6:190-192 (Bio-rad) | ∨ |
| **qPCR Validation** | |  |
| Specificity (gel, sequence, melt, or digest) | Melt | ∨ |
| For SYBR Green I, Cq of the NTC | NTC with a Cq less than 38 | ∨ |
| Standard curves with slope and y-intercept | Slope: -3.450, y-int:35.332 | ∨ |
| PCR efficiency calculated from slope | Efficiency less than 90  Efficiency greater than 110 | ∨ |
| r2 of standard curve | Std curve R2 less than 0.980 | ∨ |
| Linear dynamic range | Not Applicable | ∨ |
| Cq variation at lower limit | Not Applicable | ∨ |
| Evidence for limit of detection | Not Applicable | ∨ |
| If multiplex, efficiency and LOD of each assay. | Not Applicable | ∨ |
| **Data Analysis** | |  |
| qPCR analysis program (source, version) | Bio-rad CFX manager | ∨ |
| Cq method determination | The measured Cq values are proportional to the log base 2 (log2) of the concentration of the measured, which is a logarithmic response | ∨ |
| Outlier identification and disposition | Cq more than 3 sd from technical means (statistical outlier) | ∨ |
| Results of NTCs | Not Applicable | ∨ |
| Justification of number and choice of reference genes | Actin, encodes a major structural protein in many cell types and considered the ideal reference gene for RT-qPCR analysis and is most frequently used. Actin (AB181991) gene was used to normalize the quantification of expression  Page6:192-193 | ∨ |
| Description of normalisation method | Comparative Cq (△△Cq) method | ∨ |
| Number and stage (RT or qPCR) of technical replicates | *n* = 3, page25:927-932 | ∨ |
| Repeatability (intra-assay variation) | See error bars in Figure 8 | ∨ |
| Statistical methods for result significance | Independent Samples T-test | ∨ |
| Software (source, version) | IBM SPSS Statistics25 | ∨ |