

| ITEM TO CHECK                                             | Provided (Y/N) | CHECKLIST                                                   |
|-----------------------------------------------------------|----------------|-------------------------------------------------------------|
| <b>EXPERIMENTAL DESIGN</b>                                |                |                                                             |
| Definition of experimental and control groups             | Y              | In material and methods                                     |
| Number within each group                                  | Y              | In material and methods                                     |
| <b>SAMPLE</b>                                             |                |                                                             |
| Description                                               | Y              | In material and methods                                     |
| Microdissection or macrodissection                        | N              | N/A                                                         |
| Processing procedure                                      | Y              | In material and methods                                     |
| If frozen - how and how quickly?                          | Y              | In material and methods                                     |
| If fixed - with what, how quickly?                        | N              | N/A                                                         |
| Sample storage conditions and duration                    | Y              | In material and methods                                     |
| <b>NUCLEIC ACID EXTRACTION</b>                            |                |                                                             |
| Procedure and/or instrumentation                          | Y              | In material and methods                                     |
| Name of kit and details of any modifications              | Y              | In material and methods                                     |
| Details of DNase or RNase treatment                       | N              | In material and methods                                     |
| Contamination assessment (DNA or RNA)                     | Y              | In material and methods                                     |
| Nucleic acid quantification                               | N              | Not performed                                               |
| Instrument and method                                     | N              | Not performed                                               |
| RNA integrity method/instrument                           | N              | Not performed                                               |
| RIN/RQI or Cq of 3' and 5' transcripts                    | N              | Not performed                                               |
| Inhibition testing (Cq dilutions, spike or other)         | Y              | In material and methods                                     |
| <b>REVERSE TRANSCRIPTION</b>                              |                |                                                             |
| Complete reaction conditions                              | Y              | In material and methods                                     |
| Amount of RNA and reaction volume                         | Y              | In material and methods                                     |
| Priming oligonucleotide (if using GSP) and concentration  | Y              | In material and methods                                     |
| Reverse transcriptase and concentration                   | Y              | In material and methods                                     |
| Temperature and time                                      | Y              | provided in Supplementary material                          |
| <b>qPCR TARGET INFORMATION</b>                            |                |                                                             |
| Sequence accession number                                 | Y              | provided in Supplementary material                          |
| Amplicon length                                           | Y              | provided in Supplementary material                          |
| <i>In silico</i> specificity screen (BLAST, etc)          | N              | not provided                                                |
| Location of each primer by exon or intron (if applicable) | N              | N/A                                                         |
| What splice variants are targeted?                        | N              | N/A                                                         |
| <b>qPCR OLIGONUCLEOTIDES</b>                              |                |                                                             |
| Primer sequences                                          | Y              | provided in Supplementary material                          |
| Probe sequences                                           | Y              | provided in Supplementary material                          |
| Location and identity of any modifications                | N              | N/A                                                         |
| <b>qPCR PROTOCOL</b>                                      |                |                                                             |
| Complete reaction conditions                              | Y              | provided in Supplementary material                          |
| Reaction volume and amount of RNA                         | Y              | provided in material and methods and Supplementary material |
| Primer, (probe), Mg++ and dNTP concentrations             | Y              | provided in SI and according to kit instructions            |
| Polymerase identity and concentration                     | Y              | According to kit instructions                               |
| Buffer/kit identity and manufacturer                      | Y              | According to kit instructions                               |
| Additives (SYBR Green I, DMSO, etc.)                      | Y              | According to kit instructions                               |
| Complete thermocycling parameters                         | Y              | provided in SI                                              |
| Manufacturer of qPCR instrument                           | Y              | provided in materials and methods                           |
| <b>qPCR VALIDATION</b>                                    |                |                                                             |
| Specificity (gel, sequence, melt, or digest)              | N              | N/A                                                         |
| For SYBR Green I, Cq of the NTC                           | N              | N/A                                                         |
| Standard curves with slope and y-intercept                | Y              | provided in Supplementary material                          |
| PCR efficiency calculated from slope                      | Y              | provided in Supplementary material                          |
| R <sup>2</sup> of standard curve                          | Y              | provided in Supplementary material                          |
| Linear dynamic range                                      | N              | Not assessed                                                |
| Cq variation at lower limit                               | N              | Not determined                                              |
| Evidence for limit of detection                           | N              | N/A                                                         |
| <b>DATA ANALYSIS</b>                                      |                |                                                             |
| qPCR analysis program (source, version)                   | Y              | In material and methods                                     |
| Cq method determination                                   | Y              | In material and methods                                     |
| Outlier identification and disposition                    | N              | N/A                                                         |
| Results of NTCs                                           | Y              | provided in results section                                 |
| Justification of number and choice of reference genes     | N              | N/A                                                         |
| Description of normalisation method                       | N              | N/A                                                         |
| Number and concordance of biological replicates           | Y              | In material and methods                                     |
| Number and stage (RT or qPCR) of technical replicates     | Y              | In material and methods                                     |
| Repeatability (intra-assay variation)                     | N              | N/A                                                         |
| Statistical methods for result significance               | N              | N/A                                                         |
| Software (source, version)                                | N              | N/A                                                         |