ІТЕМ ТО СНЕСК	IMPORTANCE	CHECKLIST
EXPERIMENTAL DESIGN Definition of experimental and control groups	F	Line 249
Number within each group	E	Line 249
Assay carried out by core lab or investigator's lab? Acknowledgement of authors' contributions	D D	
SAMPLE		
Description Volume/mass of sample processed	E D	
Microdissection or macrodissection	E	Line 256
Processing procedure If frozen - how and how quickly?	E E	Line 256
If fixed - with what, how quickly? Sample storage conditions and duration (especially for FFPE samples)	E E	
NUCLEIC ACID EXTRACTION	E	line 256
Procedure and/or instrumentation Name of kit and details of any modifications	E E	Line 274
Source of additional reagents used	D	LITTIE 274
Details of DNase or RNAse treatment Contamination assessment (DNA or RNA)	E E	Line 275 Line 275
Nucleic acid quantification	E	Li ne 275
Instrument and method Purity (A260/A280)	Е D	Line 276
Yield	D	
RNA integrity method/instrument RIN/RQI or Cq of 3¹ and 5¹ transcripts	E E	Line 276 Line 276
Electrophoresis traces	D	21110 270
Inhibition testing (Cq dilutions, spike or other) REVERSE TRANSCRIPTION	E	
Complete reaction conditions	E	11
Amount of RNA and reaction volume Priming oligonucleotide (if using GSP) and concentration	E E	<u>Line 277</u> Line 277
Reverse transcriptase and concentration	E	Li ne 277
Temperature and time Manufacturer of reagents and catalogue numbers	E D	Line 277
Cqs with and without RT	D*	
Storage conditions of cDNA qPCR TARGET INFORMATION	D	
If multiplex, efficiency and LOD of each assay.	E	
Sequence accession number Location of amplicon	Е D	
Amplicon length	E	
In silico specificity screen (BLAST, etc) Pseudogenes, retropseudogenes or other homologs?	E D	
Sequence alignment	D	
Secondary structure analysis of amplicon Location of each primer by exon or intron (if applicable)	D E	
What splice variants are targeted?	E	
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences	E E	Line 282
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number	E D	Line 282
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications	E D D** E	Li ne 282 Li ne 282
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides	E D D** E D	
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RIPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Pruffication method qPCR PROTOCOL	E D D** E D	
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions	E D D** E D	Line 282
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer B Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations	E D D*** E D D E E E	Line 282 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration	E D D** E D D	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, Grobel, Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer	E D D D E E E E E D D D	Line 282 Line 279 Line 279 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dMTP concentrations Polymerase identity and concentration Buffer/kti identity and manufacturer	E D D*** E D D D E E E E E E E E	Line 282 Line 279 Line 279 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLOTIDES Primer sequences RTPrimer B Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters	E D D D E E E D D D E E E E E D D E	Line 282 Line 279 Line 279 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer DB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument	E D D*** E D D D E E E E E E E E D D D D E E E E	Line 282 Line 279 Line 279 Line 279 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLOTIDES Primer sequences RTPrimer Sequences RTPrimer B Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION	E D D D D E E D D E E	Line 282 Line 279 Line 279 Line 279 Line 279 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer Sequences RTPrimer Sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (sed, sequence, melt, or digest)	E D D D D D D D D D D D D D D D D D D D	Line 282 Line 279 Line 279 Line 279 Line 279 Line 279 Line 279
What splice variants are targeted? qPCR OLIGONUCLOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, Grobe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green J, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC	E D D D D E E D D E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer Sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope	E D D E D E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, Grobel, Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error	E D D D E E D D E E E E E E E D D E E E E E D D E E E E E E D D E E E E E E D D E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer Sequences RTPrimer Sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/bubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR Instrument qPCR VALIDATION Evidence of optimistation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range	E D D D E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of CDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Bufferklt identity and manufacturer Exact chemical constitution of the buffer Additives (SyBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Ca of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error 12 of standard curve Linear dynamic range Cq variation at lower limit	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer Sequences RTPrimer DB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR PKI instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error 12 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer Sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Bufferkit identity and manufacturer Exact chemical constitution of the buffer Additives (SyBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay.	E D D D E E E E E E E E E E E E E E E E	Line 282 Line 279
qPCR OLIGONUCLOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/bubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR PRICE instrument qPCR VAUIDATION Evidence of optimistation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay. DATA ANALYSIS JATA ANALYSIS JATA ANALYSIS JATA ANALYSIS JATA ANALYSIS JATA ANALYSIS	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer Sequences RTPrimer Sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error 12 of standard curve Linear dynamic range Cq variation at lower limit Confidence interval for PCR efficiency or standard error 12 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay. DATA ANALYSIS	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSC), etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCRVAIIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error 12 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay. DAYA ANALYSIS qPCR analysis program (source, version) Cq method determination Outlier identification and disposition	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences RTPrimer DB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCR VALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay. DAMA NANISNS qPCR analysis program (source, version) CQ method determination Outlier i detentification and disposition	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? gPCR OLIGONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method gPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Butfler/kit identity and manufacturer Exact chemical constitution of the buffer Additives SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setur (manual/robotic) Manufacturer of gPCR instrument gPCR VAILDATION To SYBR Green L, Go of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay. DATA ANALYSIS gPCR analysis program (source, version) Cq method determination Outlier identification and disposition Results of NTCS Louis and manufacturer Louis and manufacturer Linear dynamics Li	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? qPCR OLIGONUCLEOTIDES Primer sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, Iprobe). Mgg++ and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical consitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument qPCRVALIDATION Evidence of optimisation (from gradients) Specificity (gel, sequence, mell, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error 12 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence of nimit of detection If multiplex, efficiency and LOD of each assay, DYAYAMYISIS qPCR analysis program (source, version) C q method determination Outlier i detentification and disposition Results of NTCS Lustification of number and choice of reference genes Description of normalisation method Number and concordance of biological replicates	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? PCR OLICONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method QPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, probe, Mgx++ and dNTP concentrations Reaction volume and amount of cDNA/DNA Primer, probe, Mgx++ and dNTP concentrations Polymerase identity and concentration Bufferkit identity and manufacturer Exact chemical constitution of the buffer Additives SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for I mint of detection If multiplex, efficiency and LOD of each assay. DYAR ANAINSIS Outper of policy intervals of the process of	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? PCR OLICONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method QCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Bufferkit identity and manufacturer Exact chemical constitution of the buffer Additives (SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of qPCR instrument QPCR VALIDATION Evidence of optimisation (from gradients) Specificity (ge.) sequence, melt, or digest) For SYBR Green I, C. of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence intervals throughout range Evidence for limit of detection If multiplex, efficiency and LOD of each assay. DATA NALYSIS QPCR analysis program (source, version) C. q method determination Outlier identification and disposition Results of NTCS Institution on romalisation method Number and sonocrackae of biological replicates Number and stonocrackaes of sources and sources of source	E D D E E E E E E E E E E E E E E E E E	Line 282 Line 279
What splice variants are targeted? PCR OLICONUCLEOTIDES Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method QPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, probe, Mgx++ and dNTP concentrations Reaction volume and amount of cDNA/DNA Primer, probe, Mgx++ and dNTP concentrations Polymerase identity and concentration Bufferkit identity and manufacturer Exact chemical constitution of the buffer Additives SYBR Green I, DMSO, etc.) Manufacturer of plates/tubes and catalog number Complete thermocycling parameters Reaction setup (manual/robotic) Manufacturer of optimisation (from gradients) Specificity (gel, sequence, melt, or digest) For SYBR Green I, Cq of the NTC Standard curves with slope and y-intercept PCR efficiency calculated from slope Confidence interval for PCR efficiency or standard error r2 of standard curve Linear dynamic range Cq variation at lower limit Confidence intervals throughout range Evidence for I mint of detection If multiplex, efficiency and LOD of each assay. DYAR ANAINSIS Outper of policy intervals of the process of	E D D E E E E E E E E E E E E E E E E E	Line 279

Table 1. MIQE checklist for authors, reviewers and editors. All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If using primers obtained from RTPrimerDB, information on qPCR target, oligonucleotides, protocols and validation is available from that source.

^{*:} 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.

^{**:} Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information, it cannot be an essential requirement. Use of such assays is advised against.