RT-qPCR Checklist
Experimental Design

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Defined experimental and control group		☐
Number within each group		☐

RT-qPCR Checklist
Sample

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Sample descriptions provided		☐
Sample macro or micro dissection		☐
Processing procedure		☐
Check if frozen. If so, how was it frozen and how quickly?		☐
Check if fixed. If so, with what and how quickly?		☐

RT-qPCR Checklist
Nucleic Acid Extraction

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Procedure and/or instrumentation ready & described		☐
Name of kit and details of any modifications		☐
Contamination assessment (DNA or RNA)		☐
Nucleic acid quantification completed		☐
Instrument and method described		☐
RIN/RQI or Co of 3' and S' transcripts		☐
Inhibition testing (Co dilutions, spike or other) completed		☐

RT-qPCR Checklist
Reverse Transcription
Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Complete reaction conditions		☐
Amount of RNA and reaction volume defined		☐
Priming oligonucleotide (if using GPS) and concentration		☐
Reverse transcriptase and concentration		☐
Temperature and time		☐

RT-qPCR Checklist
qPCR Target Information

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
If multiplex, efficiency and LOD of each assay		☐
Sequence accession number		☐
Amplicon length		☐
In silico specificity screen (BLAST,etc)		☐
Sequence alignment		☐
Location of each primer by exon or intron (if applicable)		☐
Targeted splice variants noted		☐

RT-qPCR Checklist
Oligonucleotides

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Primer sequences		☐
Location and identity of any modifications		☐

RT-qPCR Checklist
qPCR Protocol

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Complete reaction conditions		☐
Reaction volume and amount of cDNA/DNA		☐
Primer, (probe),Mg^{2+} and dNTP concentrations		☐
Polymerase identity and concentration		☐
Buffer/kit identity and manufacturer		☐
Additives (SYBR Green I, DMSO, etc.)		☐
Complete thermocycling parameters		☐
Manufacturer of qPCR instrument		☐

RT-qPCR Checklist
qPCR Validation

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
Specificity (gel, sequence, melt, or digest)		☐
For SYBR Green I, Co of the NTC		☐
Standard curves with slope and y-intercept		☐
PCR efficiency calculated from slope		☐
R2 of standard curve		☐
Linear dynamic range		☐
Cq variation at lower limit		☐
Evidence for limit of detection		☐
If multiplex, efficiency and LOD of each assay		☐

RT-qPCR Checklist
Data Analysis

Check off items once complete or ready

	Description of the step (builds your materials & methods section)	*Check ✓*
qPCR analysis program (source, version)		☐
Cq method determination		☐
Outlier identification and disposition		☐
Results of NTCs (No template controls)		☐
Justification of number and choice of reference genes		☐
Number and stage (RT or qPCR) of technical replicates		☐
Repeatability (intra-assay variation)		☐
Statistical methods for result significance		☐
Software (source, version)		☐

References

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