

**Supplemental Table 3.** 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.

ITEM TO CHECK	IMPORTANCE	COMMENTS
<b>EXPERIMENTAL DESIGN</b>		
Definition of experimental and control groups	E	TCDD-exposed and vehicle (DMSO)
Number within each group	E	5
Assay carried out by core lab or investigator's lab?	D	Investigator's lab
Acknowledgement of authors' contributions	D	Yes
<b>SAMPLE</b>		
Description	E	Testis from adult zebrafish exposed to sublethal levels of TCDD
Volume/mass of sample processed	D	
Microdissection or macrodissection	E	Microdissection
Processing procedure	E	Zebrafish tissues were collected and immediately frozen in liquid nitrogen
If frozen - how and how quickly?	E	Immediate
If fixed - with what, how quickly?	E	No
Sample storage conditions and duration	E	-80°C
<b>NUCLEIC ACID EXTRACTION</b>		
Procedure and/or instrumentation	E	Total RNA was extracted using the QIAGEN RNeasy mini kit following the manufacturer's protocol. Tissue was homogenized in Qiazol.
Name of kit and details of any modifications	E	QIAGEN RNeasy mini kit per manufacturer's protocol
Source of additional reagents used	D	
Details of DNase or RNase treatment	E	N/A. RNeasy silicamembrane technology efficiently removes most of the DNA without DNase treatment. All plastic and reagents used are certified RNase free.
Contamination assessment (DNA or RNA)	E	No reverse transcription reaction controls were run with qPCR probes
Nucleic acid quantification	E	RNA quantity was measured on Nanodrop-1000
Instrument and method	E	RNA quantity was measured on Nanodrop-1000 following manufacturer's protocol
Purity (A260/A280)	D	See Supplemental Table 1
Yield	D	
RNA integrity method/instrument	E	Agilent Bioanalyzer
RIN/RQI or Cq of 3' and 5' transcripts	E	See Supplemental Table 1
Electrophoresis traces	D	
Inhibition testing (Cq dilutions, spike or other)	E	Not performed. Amplification product for housekeeping gene assay sufficient to rule out the presence of inhibitors of PCR and reverse-transcriptase inhibitors.
<b>REVERSE TRANSCRIPTION</b>		
Complete reaction conditions	E	Reverse Transcription reaction carried out with the High Capacity cDNA Reverse Transcription Kit following manufacturer's protocol
Amount of RNA and reaction volume	E	Amount of RNA: 500 ng; Reaction volume: 20 µl
Priming oligonucleotide (if using GSP) and concentration	E	N/A
Reverse transcriptase and concentration	E	High Capacity cDNA Reverse Transcription Kit components
Temperature and time	E	Manufacturer's protocol
Manufacturer of reagents and catalogue numbers	D	
Cqs with and without RT	D*	
Storage conditions of cDNA	D	
<b>qPCR TARGET INFORMATION</b>		
If multiplex, efficiency and LOD of each assay.	E	N/A
Sequence accession number	E	See Supplemental Table 4
Location of amplicon	D	
Amplicon length	E	See Supplemental Table 4
<i>In silico</i> specificity screen (BLAST, etc.)	E	All Taqman assays have been designed through Thermo Fisher's validated bioinformatics pipeline
Pseudogenes, retropseudogenes or other homologs?	D	
Sequence alignment	D	
Secondary structure analysis of amplicon	D	
Location of each primer by exon or intron (if applicable)	E	See Supplemental Table 4
What splice variants are targeted?	E	None (See Supplemental Table 4)
<b>qPCR OLIGONUCLEOTIDES</b>		
Primer sequences	E	Not available due to intellectual property of Thermo Fisher
RTPrimerDB Identification Number	D	
Probe sequences	D**	
Location and identity of any modifications	E	See Supplemental Table 4
Manufacturer of oligonucleotides	D	
Purification method	D	
<b>qPCR PROTOCOL</b>		
Complete reaction conditions	E	See materials and methods
Reaction volume and amount of cDNA/DNA	E	Reaction volume: 20 µl; cDNA amount: 2 µl
Primer, (probe), Mg <sup>++</sup> and dNTP concentrations	E	Primer concentration in 20x mix: 900nm; Probe concentration in 20x mix: 150 nm; Mg <sup>++</sup> and dNTP (in Taqman universal master mix no Amperase UNG) concentration unavailable due to intellectual property of Thermo Fisher
Polymerase identity and concentration	E	Amplitaq Gold DNA polymerase. Concentration unavailable
Buffer/kit identity and manufacturer	E	Taqman universal master mix no Amperase UNG by Thermo Fisher
Exact chemical constitution of the buffer	D	
Additives (SYBR Green I, DMSO, etc.)	E	None
Manufacturer of plates/tubes and catalog number	D	
Complete thermocycling parameters	E	50°C for 2 minutes, 90°C for 10 minutes, 40 cycles of 95°C for 15 seconds and 60°C for 1 minute
Reaction setup (manual/robotic)	D	Robotic
Manufacturer of qPCR instrument	E	Applied Biosystems 7900

<b>qPCR VALIDATION</b>		
Evidence of optimization (from gradients)	D	
Specificity (gel, sequence, melt, or digest)	E	
For SYBR Green I, Cq of the NTC	E	
Standard curves with slope and y-intercept	E	
PCR efficiency calculated from slope	E	
Confidence interval for PCR efficiency or standard error	D	
r2 of standard curve	E	
Linear dynamic range	E	
Cq variation at lower limit	E	
Confidence intervals throughout range	D	
Evidence for limit of detection	E	
If multiplex, efficiency and LOD of each assay.	E	All primers and probes were supplied by Thermo Fisher Scientific. Taqman probes have been prevalidated. <sup>(17)</sup>
<b>DATA ANALYSIS</b>		
qPCR analysis program (source, version)	E	Sequence Detection System 2.4
Cq method determination	E	Cycle Threshold
Outlier identification and disposition	E	Runs were performed in triplicate and variations were shown to be minimal
Results of NTCs	E	NTCs were shown to have undetermined Cts
Justification of number and choice of reference genes	E	Actb was chosen as the reference gene. Actb was unchanged on the microarray. As we have previously used actb as a housekeeping gene, it was chosen to maintain consistency.
Description of normalization method	E	Data normalization was carried out via the $\Delta\Delta C_t$ method using actb as a housekeeping gene.
Number and concordance of biological replicates	D	
Number and stage (RT or qPCR) of technical replicates	E	qPCR reactions were done in triplicate
Repeatability (intra-assay variation)	E	Intra-experiment variation was assessed by triplicates
Reproducibility (inter-assay variation, %CV)	D	
Power analysis	D	
Statistical methods for result significance	E	Student's T-Test
Software (source, version)	E	Microsoft Excel 2013
Cq or raw data submission using RDML	D	