ITEM TO CHECK EXPERIMENTAL DESIGN	IMPORTANCE	CHECKLIST
Definition of experimental and control groups	Е	Rat H9c2 cardiac cells were cultured under normoxia during 48h (Normoxia). In parallel, the cells were subjected to Hypoxia/starvation (24h), then reoxygenated and treated with DMSO as
		vehicle control (HS/R) or with 15µM fisetin (HS/R + F) during 24h.
Number within each group Assay carried out by core lab or investigator's lab?	E D	1159
Acknowledgement of authors' contributions SAMPUE	D D	
Description	E	Rat H9c2 cardiac cells (ATCC® CRL-1446™) were purchased from ATCC (Rockville, MD, USA).
Volume/mass of sample processed Microdissection or macrodissection	D E	From 1 to 5 x 10 ⁶ H9C2 cells were harvested following drug treatment. N/A
Processing procedure	E	Cells were washed, counted in PBS (without Ca** and Mg**), centrifuged, resuspended in 1mL TRI Reagent®, snap-frozen and then stored at -80°C.
If frozen - how and how quickly? If fixed - with what, how quickly?	E E	Samples were snap-frozen in TRI Reagent® and stored at -80°C. N/A
Sample storage conditions and duration (especially for FFPE samples) NUCLEIC ACID EXTRACTION	E	Samples were stored in TRI Reagent® at -80°C until RNA extraction.
Procedure and/or instrumentation	E	Total RNA was extracted from 1 to 5 x 10 th cells with a TRI Reagent® (Sigma-Aldrich) isolation protocol. Aqueous phase was isolated with Phase lock gel-Heavy (5 Prime, Gaithersburg, MD) Total RNA was precipitated with 100% isopropanol and purified with a RNeasy® Mini kit combined with an on-column DNase treatment following the manufacturer's instructions(Clagen, Valencia, CA).
Name of kit and details of any modifications Source of additional reagents used	E D	TRI Reagent® - RNeasy® Mini kit combined with an on-column DNase treatment following the manufacturer's instructions. Chloroform (Merck); ispropanol (Merk); Ethanol (Merck); Nuclease free water (Life Technologies)
Details of DNase or RNAse treatment	E	RNeasy® Mini kit combined with an on-column DNase treatment following the manufacturer's instructions.
Contamination assessment (DNA or RNA) Nucleic acid quantification	E	DNase treatment + Bioanalyzer + primers flanking intron + Negative control (RT-PCR & qPCR) Nanodrop
Instrument and method Purity (A260/A280)	E D	Nanodrop All RNA sample: Purity (A260/A280) = 2
Yield	D E	N/A
RNA integrity method/instrument RIN/RQI or Cq of 3' and 5' transcripts	E	Bioanalyzer All RNA sample : RIN ≥ 9
Electrophoresis traces Inhibition testing (Cq dilutions, spike or other)	D E	N/A Inhibition tested with a serial dilution method to monitor linearity of Cq values.
Inhibition testing (Cq dilutions, spike or other) REVERSE TRANSCRIPTION		
Complete reaction conditions	E	I to g d RNA were reverse transcribed into cDNA using the SuperScript III (Invitrogen, Carlsbad, CA) reverse transcriptase with the following protocic RNAs were mixed with random primers, oligo (fr)12-18 and offMPF is in a total volume of 13µL Samples were heated to 65° for 5 min and incubated on ice for at least min. Then the SRY To Uffer, DTT, RNASeOUT and SQUPSCript III was added to a total volume of 20µL RT was allowed at 50°C for 60 min, and was followed by enzyme inactivation at 70°C for 15 min. Final concentrations were: 100ng of oligo(dT)12-18, 50ng of random primers, 0.5mM ANTPS_ 50mM STRP_50 MINT Final (T, BMASEOUT and 2000) of SuperScript III. To remove RNA complementary to the cDNA, 2U of Ecoli RNAseH was added and incubated at 37°C for 20 minutes. In each RT-PCR a no template control (no RNA in RT) were performed.
Amount of RNA and reaction volume	E	1µg RNA / 20µl reaction volume Produm primon: 2 Rould (Nicol CT) - Facility (final concentration)
Priming oligonucleotide (if using GSP) and concentration Reverse transcriptase and concentration	E E	Random primers : 2.5.ng/µl Oligo(dT) _{12:18} : 5.ng/µl (final concentration) SuperScript III (Invitrogen) : 10U/µl (final concentration)
Temperature and time	E	RNAs were mixed with random primers, oligo (d7)12-18 and dNTPs in a total volume of 13µL Samples were heated to 65°C for 5 min and incubated on ice for at least firmin. Then the 5X RT buffer, DTT, RNAssoQUT and SuperSprictful times added to a total volume of 12µL RT was allowed at 50°C for 50 min, and was followed ryome inactivation at 70°C for 15 min. Then the 5X RT RNAS of the 12µL RT was allowed at 50°C for 20 minutes. RNA complementary to the cDNA. 2U of Ecoli. RNased was added and incubated at 3°C for 20 minutes. Like Technologies. SuperSprictful (ECI. 18080-045), (10g0T) _{1,320} primer (1818-012), Random primers (Cat. 48190-011), 10mM dNTP Mix (18427-013), RNaseOUT 40UJul (10777-019),
Manufacturer of reagents and catalogue numbers	D	E.coli RNaseH (AM2293)
Cqs with and without RT Storage conditions of cDNA	D*	N/A - DNase treatment + primers flanking intron + Melt Curve
qPCR TARGET INFORMATION If multiplex, efficiency and LOD of each assay.	E	N/A
Sequence accession number	E	see additional MIQE-A
Location of amplicon Amplicon length	D E	see additional MIDE-A see additional MIDE-A
In silico specificity screen (BLAST, etc) Pseudogenes, retropseudogenes or other homologs?	E D	Beacon Designer Pro 8.10 software (Premier Biosoft) + NCBI BLAST tool
Sequence alignment	D D	
Secondary structure analysis of amplicon Location of each primer by exon or intron (if applicable)	E	see additional MIOE-A
What splice variants are targeted?	E	see additional MIQE-A cf. Accession number
qPCR OLIGONUCLEOTIDES		
qPCR OLIGONUCLEOTIDES Primer sequences TPTPrimer/Dl Identification Number	E D	see additional MIQE-A
Primer sequences RTPrimerDB Identification Number Probe sequences	D	N/A N/A
Primer sequences RTPrimerDB (lentification Number Probe sequences Location and identity of any modifications Manufacturer oligionucleoides	D	NIA NIA NIA EUROGENTEC (Serging, Belgium)
Primer sequences RTPrimerDB Identification Number Probe sequences Location and identity of any modifications	D**	NIA NIA NIA SISA EUROGENTEC (Seraina, Belgium) RP-Cartridoe - Gold
Primer sequences RFTPrimerD & Member Probe sequences Location and identity of any modifications Manufacturer of disprusceotides Purification method gPCR PROTOCOL Complete reaction conditions	D D D D D D D D D D D D D D D D D D D	N/A
Primer sequences RTPrimerDE Identification Number Probe sequences Probe sequences Manufacturer of disprayable of the Sequences Purification method qPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations	D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A N/A N/A N/A SUROGENTEC (Seraina, Belgium) RP-Cartridose - Gold cDN/As obtained from RT-PCR of RNA were diluted 10-fold and 2µL were mixed with SoAdvanced** Universal SYBR® Green Supermix (Bio-Rad, Nazareth, Belgium) to a final volume of 10µL containing 300mM of each primer. Amplification was carried out in the Viai***/real-lime PCR 384-well system (Applied Biosystems**) under the following conditions: heating for 3 minutes at 95°C. 40 cycles of denaburation for 15 seconds at 95°C, followed by an annealing/seteration for 1 min. After each run, a melting curve was performed with continuous temperature ramping at 0.05°C/s from 55°C to 95°C. A negative control without cDNA template was not in every assay and measures were performed in triplicates. 2µ-cDNA diluted 10 fold / 10µl reaction volume
Primer sequences RTPrimerD liberatification Number Probe sequences Location and identity of any modifications Manufacturer of origonucteoides GEGEROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentrations	E E E E E E E E E E E E E E E E E E E	N/A N/A N/A N/A N/A N/A N/A EUROSENTEC, (Seraina, Belgium) EUROSENTEC, (Seraina, Belgium) RP-Cartridor - Gold CDNAs obtained from RT-PCR of RNA were diluted 10-fold and 2µL were mixed with SsoAdvanced™ Universal SYBR® Green Supermix (Bio-Rad, Nazareth, Belgium) to a final volume of 10µL containing 300mM of each primer. Amplification was carried out in the Viain™ real-time PCR 384-well systems (Applied Bioxystems **) under the following conditions: heating for 3 minutes at 90°C. 40 cycles of centraturation for 15 seconds at 95°C, blowed by an annealing/injectersion for 1 min. After each run, a melting curve was performed with continuous temperature ramping at 0.05°Cs from 55°C to 95°C. A negative control without cDNA template was run in every assay and measures were performed in triplicates. 2µ-CARD. Additated 10 61 / 10µl reaction volume 300mM of each primer + SsaAdvanaced** Universal SYBR®-Green Supermix Sso7 flastop oplymerases
Primer sequences RTPrimerD blemfitration Number Probe sequences Location and identity of any modifications Manufacturer of oligorucideoides Politication media GEGR RG10500 Complete reaction conditions Reaction volume and amount of cDNADNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Bufferkit identity and concentration Bufferkit identity and manufacturer Exact chemical constitution of the buffer	D D D D D D D D D D D D D D D D D D D	N/A
Primer sequences RTPrimerDE Mentification Number Probe sequences Probe sequences RTPrimerDE Mentification Number Probe sequences Reaction sequences Purification method GEOR PROTOCOL Complete reaction conditions Reaction volume and amount of cONA/DNA Primer, Eprobel, Mg++ and dNTP concentrations Polymerase identity and concentration Polymerase identity and concentration Polymerase identity and concentration	E E E E E E E E	N/A
Primer sequences (RTPrimerD B Identification Number (Probe sequences (Prob	D D E E E E E E E E E E E E E E E E E E	N/A
Primer sequences RTPrimerDB (bentification Number Probe sequences Location and sidentity of any modifications Location and sidentity of any modifications Location and sidentity of any modifications Purification method OPER PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, (probe), Mg++ and oNTP concentrations Polymerase sidentity and manufacturer Exact chemical constitution of the buffer Additional Constitution of the buffer Complete thermocycling parameters Complete thermocycling parameters Reaction setting (primeruly) and calling number Complete thermocycling parameters Reaction setting (primarular/bottor)	D D" E D D E E E E E E E D D E D D D D E D D D	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences (RTPrimerD B Identification Number (Probe sequences (Prob	D D E E E E E E E E E E E E E E E E E E	N/A
Primer sequences (RTPmmetD blessification Number (Probe sequences)	D D" E D D E E E E E E E D D E D D D D E D D D	N/A
Primer sequences RTPrimerDE Identification Number Probe sequences Location and defently of any modifications Location and defently of any modifications Location and defently of any modifications Purification method GPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, probely, Mg+ and oNTP concentrations Polymerase identify and concentration Bollinath cloratily and manufacture Bollinath cloratily and manufacture Additives (CPSR Green LDNS), ortic Manufacturer of plateshabes and catalog number Complete hermocycling parameters Reaction setty (manual/robotic) Manufacturer of plateshabes and catalog number Complete hermocycling parameters Reaction setty (manual/robotic) Manufacturer of pCR instrument GRCR WAIDATION Evidence of optimisation (from gradients) Evidence of optimisation (from gradients)	D D" E D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A N/A N/A N/A BUROCRITEC, (Seraina, Belgium) BUROCRITEC, (Seraina, Belgium) RP-Cartridge - Gold CDNAs obtained from RT-PCR of RNA were diluted 10-fold and 2yL were mixed with Ssod/vanced** Universal SYBR® Green Supermix (Bic-Rad, Nazareth, Belgium) to a final volume of 10yL containing 300mM of each primer. Amplification was carried out in the Viai**7* real-time PCR 384-well system (Applied Bionystems**) under the following conditions: heating for 3 minutes at 90.5°C. 40 cycles of dentanization for 15 esconds at 95°C, blowed by an annealing/electrical for 1 min. After each run, a melting curve was performed with continuous temperature ramping at 0.05°Cs from 55°C to 95°C. A regainer control without cDNA template was not in every assay and measures were performed in tiplicates. 2yL-MA dituted 10 fold 1 full reaction volume. 300mM of each primer + Sead-Vanced** Universal SYBR® Green Supermix 300mM of each primer + Sead-Vanced** Universal SYBR® Green Supermix Ssof-Mayoncod** Universal SYBR® Green Supermix (Bionad, Nazareth, Belgium) (Cat. 172-5275) N/A Heating for 3 minutes at 95°C, 40 cycles : denaturation for 15 seconds at 95°C, fellowed by an annealing/extension for 1 min. After each run, a melting curve was performed with continuous temperature rampins at 0.05°Cs from 55°C to 95°C. CP306 thermal cycler (BioRad) / Viai** 7* real-time PCR 384-well system. (Applied Biosystems**) qPCR efficiency and experiment gendered in the CF338 thermal cycler (BioRad) / Viai*** real-time PCR 384-well system. (Applied Biosystems**) QPCR efficiency and experiment is enformed in the CF338 thermal cycler (BioRad) / Viai*** real-time PCR 384-well system. (Applied Biosystems***) No template control ICTI (To co DNA in a PCR) and for each eachy. No template control ICTI (To co DNA in a PCR) and for each eachy. No template control ICTI (To co DNA in a PCR) and for each eachy.
Primer sequences RTPrimerDE Identification Number Probe sequences Location and identify of any modifications Location and identify of any modifications Location and identify of any modifications Purification method GPCR PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, proble), Mg+ and oNTP concentrations Polymerase identify and concentration Exact Chemical Constitution of the buffer Additives (SPKR Green L DNS), etc.) Manufacturer of plates/hubbes and catalog number Complete hermocycling parameters Reaction setup (manual/robotic) Manufacturer of polymerase identification Evidence of optimisation (from gradients) Evidence of optimisation (from gradients) For SYBR Green L Q of the NTC Standard Curves with stope and y-intercept	D D'' E D D D D D D D D D D D D D D D D D D D	N/A
Primer sequences RTPrimerDE Identification Number Probe sequences Location and defently of any modifications Location and defently of any modifications Location and defently of any modifications Purification method OPER PROTOCOL Complete reaction conditions Reaction volume and amount of cDNA/DNA Primer, probely, Mg++ and dNTP concentrations Polymerase identify and consumeration Buffecht cleritify and manufacturer Additives (SYRS Green LDMSO, etc.) Manufacturer of plateshubes and catalog number Complete hermocycling parameters Reaction setup (manual/robotic) Manufacturer of oPCR instrument GSCR VALIDATION Evidence of optimisation (from gradients) For SYRS Green LO q of the NTC Standard Curves with slope and vintercept PCR efficiency calculated from slope Confidence interval to PCR efficiency or standard error	D D D D D D D D D D D D D D D D D D D	N/A
Primer sequences (RTPmmetD blessification Number (Probe sequences) (RTPmmetD blessification Number (Probe sequences) (RTPmmetD blessifications) (Residence of digramy modifications) (Residence of digramy blessifications) (Residence of digramy blessifica	D	N/A
Primer acquences RTPrimerDE Membrate Probe sequences RTPrimerDE Membrate Probe sequences Runding Membrate Membrate of big dray modifications Manufacturer of big dray modifications Manufacturer of big dray modifications Rection returns of the sequence of	D D D E E E E E E E E E E E E E E E E E	N/A
Primer sequences RTPrimerDE Identification Number Probe sequences RTPrimerDE Identification Number Probe sequences Runding and a sequences Runding and a sequences Runding and a sequences Runding and	D D D E E E E E E E E E E E E E E E E E	N/A
Primer sequences RTPrimerDE Identification Number Probe sequences RTPrimerDE Identification Number Probe sequences Runder of digramy endifications Manufacturer of digramy endifications Manufacturer of digramy endifications Reaction method qCCR PROTOCOL Compiler reaction conditions Reaction volume and amount of cONAIDNA Primer, (probe). Mg++ and dNTP concentrations Polymerase identity and concentration Bufferful identity and manufacturer Exact chemical constitution of the buffer Additives (PSR Green L DNS), dtc) Manufacturer of platerfulbes and catalog number Compiler thermocyling parameters Reaction setup (manufactocit) Manufacturer of politerfulbes and catalog number Compiler thermocyling parameters Reaction setup (manufactocit) Manufacturer of politerfulbes and catalog number Compiler deprending of the compiler of t	D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences RTPrimerDE Identification Number Probe sequences RTPrimerDE Identification Number Probe sequences Runding and a sequences Runding and a sequences Runding and a sequences Runding and	D D D E E E E E E E E E E E E E E E E E	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences (RTPrimerDE Identification Number (RTPrimerDE Identification Number (RTPrimerDE Identification Number (RTPrimerDE Identification Identification) (Abundanturer of digranucleotides (Rundanturer of digranucleotides (R	D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences (RTPrimerDE) Elemification Number (Probe sequences (Probe	D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences RTPrimerDE Membrater Probe sequences RTPrimerDE Membrater Probe sequences Runding Membrater Residence of oligany modifications Manufacturer of oligany modifications Manufacturer of oligany modifications Reaction wature and amount of cONADNA Primer (probe). Mg-+ and dNF concentrations Primer (probe). Mg-+ and dNF concentrations Polymerasa identity and concentration Butlerfact is other of concentration Pulmerasa identity and concentration Pulmerasa identity and manufacturer Exact chemical constitution of the buffer Additives (PSR Green I, MSD, etc.) Manufacturer of plates/bubbs and catalog number Complete hermocyling parameters Reaction setup (manufacturic) Manufacturer of PoR instrument QPCR VALIDATION Sylvanory of the Control of the NTC Standerd curves with stope and y-intercept PGR deficiency acclusited from stope Confidence intervals throughout range Confidence intervals thr	D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A EUROCENTEC. Charina. Belgium) EUROCENTEC. Charina. Belgium) EUROCENTEC. Charina. Belgium) EUROCENTEC. Charina. Belgium) CDNAs obtained from RT-PCR of RNA were diluted 10-fold and 2µL were mixed with Sood-dvanoed*** Universal SYBR® Green Supermix (Bio-Rad, Nazareth, Belgium) to a final volume of 10µL containing 300mM of each primer. Amplification was carried out in the Viai***/ real-time PCR 384-well system (Applied Biosystems**) under the Biolowing conditions: healing for 3 minutes at 95°C. 40 cycles of denaturation for 15 seconds at 95°C, blowed by an annealing/leatersion for 1 min. After each run, a melting curve was performed with continuous temperature ramping at 0.05°Cs from 55°C to 95°C. A regaine control without CDNA template was not in every sassy and measures were performed in tiplicates. 2u-CDNA ditalest of 10x4 (1.04) reactions of viaine at 300mM of each primer + Sead-Avenaced** Universal SYBR® Green Supermix (Biorad, Nazareth, Belgium) (Cat. 172-5275) SasoAdvanoed** Universal SYBR® Green Supermix (Biorad, Nazareth, Belgium) (Cat. 172-5275) NA Heating for 3 minutes at 95°C. 40 cycles : denaturation for 15 seconds at 95°C, fellowed by an annealing/leatersion for 1 min. After each run, a melting curve was performed with continuous temperature ramping at 0.05°Cs from 55°C to 95°C. CPX80 thermal cycler (BioRad) / Viai*** real-time PCR 384-well system (Applied Biosystems**) Temperature gradient performed in the CFX86 thermal cycler (BioRad) / Viai*** real-time PCR 384-well system. (Applied Biosystems**) Gene-specific amplification confirmed by a single board in 4% E-Gef** (Life technologies). No templication signal detected sea detilicional MiOC-A sea d
Primer sequences RTPrimerDB Identification Number Probe sequences Probe sequences Probe sequences Probe sequences Reaction with a proper sequence of sequences Purification method qCR RROTOCOL Complete reaction conditions Reaction volume and amount of cDNADNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identify and concentration BufferMs identify and manufacturer Exact chemical consolution of the buffer Additives (2018 Center LDMSC) Reaction of the buffer Additives (2018 Center LDMSC) Reaction setup (manufacturer) Polymeration setup (manufacturer) Reaction setup (manufacturer) Polymeration setup (manufacturer) Reaction setup (manufacturer) Polymeration setup (manufacturer) Reaction curves Linear dynamic seculation from slope Cq variation at lower limit Reaction for limit of detection QPCR analysis program (source, version) Cq method determination Outlier identification and disposition Results of NTCS Justification of number and choice of reference genes	D. D	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences RETPIEMED Electrification Number Probe sequences Probe sequences Residences Reside	D D D D D D D D D D D D D D D D D D D	N/A N/A N/A N/A N/A N/A N/A N/A
Primer sequences RTPrimerDB Identification Number Probe sequences RTPrimerDB Identification Number Probe sequences Reaction sequences Reaction method qCR RROTOCOL Complete reaction conditions Reaction volume and amount of cDNADNA Primer, (probe), Mg++ and dNTP concentrations Polymerase identity and concentration Bufferficit identify and manufacturer Exact chemical constitution of the buffer Additives (SPR Green L MSQ, etc.) Manufacturer of plates/tubes and caladion number Complete harmonyching parameters Reaction setup (in qnucla richard to the setup of the	D D D D D D D D D D D D D D D D D D D	NA N
Primer sequences RETPIEMED Electrification Number Probe sequences Probe sequences Residences Reside	D D D D D D D D D D D D D D D D D D D	NA N
Primer sequences IFTrimerab Bisentification Number Probe sequences First was a proper or provided to the provided to the provided to the primerab provided to the primerab pri	D. D	N/A N/A EUROCENTE Currains. Belgium) BP-Carrains. Belgium) CDNAs obtained from RT-PCR of RNA were diluted 10-fold and 2µt, were mixed with 5sod-dwanced** Universal SYBR® Green Supermix (Bio-Rad, Nazareth, Belgium) to a final volume of 10µt, containing 300mM of each primer. Amplification was carried out in the vitia** 7 real-time PCR 384-well system (Applied Biosystems**) under the following condisions: healing for 3 minutes at 50°C, 40 cycles of dentaination for 15 seconds at 95°C, 100mb by an annealing-detension for 1 min. After each run, a retirent curve was predicted with continuous temperature ramping at 0.05°Cs from 55°C to 95°C. A negative control without cDNA template was run in every assay and measures were performed in thiplicates. 30.01 Additional To 10 Additional 10 Add 1 Students 10 Additional 10 Add
Primer sequences IFT Primer Del Benstification Number Frobe sequences Frobe sequences Frobe sequences Profes sequences Profes sequences Purification method del Complete reaction conditions Reaction volume and amount of cDNADNA Primer, profes, Mg-+ and dNTP concentrations Polymerase identify and concentration Bufferfald selentify and concentration Polymerase identify and concentration Bufferfald selentify and manufacturer Exact chemical constitution of the buffer Additives (SYR Green LDMS), dtc) Manufacturer of plates/tubes and caladiog number Complete thermocyling parameters Reaction setup (manual/robotic) Manufacturer of plates/tubes and caladiog number Complete thermocyling parameters Reaction setup (manual/robotic) Manufacturer of plates/tubes and caladiog number Complete thermocyling parameters Reaction setup (manual/robotic) Manufacturer of plates/tubes and caladiog number Complete thermocyling parameters Reaction setup (manual/robotic) Manufacturer of plates/tubes and caladiog number Complete on the plates/tubes and caladiog number Por SYBR Green LO (Complete thermocyling parameters Specificity (gal. sequence, melt, or digest) For SYBR Green LO (Complete thermocyling parameters) Por SYBR Green LO (Complete thermocyling parameters Por SYBR Green LO (Complete thermocyling parameters) Confidence interval for PCR efficiency or standard error 2 of standard curves with slope and y-intercept PCR efficiency adulated from slope Confidence interval for PCR efficiency or standard error 2 of standard curve Linear dynamic range Evidence for limit of detection Complete for the melting and Complete and choice of reference genes Description of normalisation method Number and concordance of biological replicates Repostability (forter-assay variation) Repostabilit	D D D D D D D D D D D D D D D D D D D	NA NA NA NA NA NA NA EURODENTEC, (Sereino, Bedgum) EURODENTEC, (Sereino, Bedgum) RPC-attridors - Gold RPC-attridors - Gold RPC-attridors - Gold CDNAs obtained from RT-PCR of RNA were diluted 10-fold and 2pL were mixed with Sood-dvanced*** Universal SYBR® Green Supermix (Bio-Rad, Nazareth, Belgium) to a final volume of 10pL containing 300mM of each primer. Amplification was carried out in the Via**** Treal-time PCR 384-well system (Applied Biosystems**) under the following conditions: healing for 3 minutes at 95°C. 40 cycles is cleanabilities for 15 for 1 min., After each in it in place is the viame of 10pL containing 300mM of each primer at 15 seconds at 95°C, biolowed by an annealing/leatens for 1 min., After each un. a melting curve was performed in the viame of 15 seconds at 95°C, biolowed by an annealing/leatens for 1 min. After each un. a melting curve was performed with continuous temperature araping at 10 seconds at 95°C, biolowed by an annealing/leatension for 1 min. After each un. a melting curve was performed with continuous temperature araping at 0.05°Cs from 55°C to 95°C. CPS® themself of 3 minutes at 95°C, 40 cycles : denabration for 15 seconds at 95°C, biolowed by an annealing/leatension for 1 min. After each un. a melting curve was performed with continuous temperature ramping at 0.05°Cs from 55°C to 95°C. CPS® themself cycler (BioRad) / Via*** 7 real-time PCR 384-well system (Applied Biosystems**) Gene-specific amplification confirmed by a single band in 45°C. Fold** (Life technologies). Melt Curve analysis performed in each assay. No template control INTC! (no cDNA in pRCR 384-well system; (Applied Biosystems**) Gene-specific amplification confirmed by a single band in 45°C. Fold** (Life technologies). Melt Curve analysis performed in each assay. No template control INTC! (no cDNA in pRCR 364 well system in problems and problems and problems and problems and problems and problems and problems are additional MIOC-A see additional MIOC-A see additional MIOC-A see additional MIOC-A see ad

Table 1. MIOE 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 RTPrimeriOB, information on gPCR target, oligonucleoides, 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.