MIQE checklist Item to check	Importance	Included?	Comments
Experimental design	importance	menuaeur	
Experimental design Definition of experimental and control groups	E	Yes	
Number within each group	E	Yes	
Assay carried out by the core or investigator's laboratory?	D	Yes	Investigator's lab
Acknowledgment of authors' contributions	D	Yes	
Sample			
Description	E	Yes	
Volume/mass of sample processed	D F	Yes	
Microdissection or macrodissection Processing procedure	F	No No	n.a. n.a.
If frozen, how and how quickly?	E	No	n.a.
If fixed, with what and how quickly?	E	No	n.a.
Sample storage conditions and duration (especially for FFPEb samples)	E	Yes	All samples stored at -20°C
Nucleic acid extraction			
Procedure and/or instrumentation	E	Yes	
Name of kit and details of any modifications Source of additional reagents used	E	Yes Yes	
Details of DNase or RNase treatment	F	No	n.a.
Contamination assessment (DNA or RNA)	E	No	n.a.
Nucleic acid quantification	E	Yes	
Instrument and method	E	Yes	
Purity (A260/A280)	D	No	n.a.
Yield	D	No	n.a.
RNA integrity: method/instrument RIN/RQI or Cq of 3. and 5. transcripts	E	No No	n.a. n.a.
Electrophoresis traces	D	No	n.a.
nhibition testing (Cq dilutions, spike, or other)	E	Yes	Cq dilutions
Reverse transcription			
Complete reaction conditions	E	No	n.a.
Amount of RNA and reaction volume	E	No	n.a.
Priming oligonucleotide (if using GSP) and concentration	E	No	n.a.
Reverse transcriptase and concentration Temperature and time	E	No No	n.a. n.a.
Manufacturer of reagents and catalogue numbers	D	No	n.a.
Cqs with and without reverse transcription	D	No	n.a.
Storage conditions of cDNA	D	No	n.a.
qPCR target information			
Gene symbol	E	Yes	HMGA1. n.a. for MON810 target
Sequence accession number	E	Yes	AJ131373 (hmg), AF434709 (MON810)
			719 - 797 (intron 4 on acc. AJ131373 -hmg), 767 - 868 (junction region on acc.
Location of amplicon Amplicon length	D	No Yes	AF434709 -MON810) 79bp (hmg), 92 bp (MON810)
Amplicon length	C	res	/ 30p (mng), 32 0p (MON810)
			Blastn: amplicon aligns only on Zea mays hmgA gene (AJ131373) and a reference
			plasmid (JX434027) (hmg assay), and on transgenic maize (acc. No JQ406879 and
In silico specificity screen (BLAST, and so on)	E	Yes	AF434709) (MON810 assay)
Pseudogenes, retropseudogenes, or other homologs?	D	Yes	not found
Sequence alignment	D	No	
			mfold applysis of target sequence (EO°C, 2.5 mM Mg2), EO mM No.), 7 structures with
			mfold analysis of target sequence (50°C, 2.5 mM Mg2+, 50 mM Na+): 7 structures wi deltaG from = -0.88 to -0.07 kcal/mol (for hmg) and two structures with deltaG from
Secondary structure analysis of amplicon	D	Yes	3.89 to -3.63 kcal/mol (for MON810)
Location of each primer by exon or intron (if applicable)	E	Yes	Both primers on intron 4 of acc. AJ131373 (hmg)
What splice variants are targeted?	E	No	n.a.
qPCR oligonucleotides			
Primer sequences	E	Yes	
			n.a.
RTPrimerDB identification number	D	No	
		No Yes	
Probe sequences	D D	Yes	
Probe sequences Location and identity of any modifications		No Yes Yes Yes	Modification of the quenchers (BHQ1) Eurofins MWG Operon
Probe sequences .ocation and identity of any modifications Manufacturer of oligonucleotides	D D E	Yes Yes	Modification of the quenchers (BHQ1)
Probe sequences Location and identity of any modifications Vlanufacturer of oligonucleotides Purification method	D D E D	Yes Yes Yes	Modification of the quenchers (BHQ1) Eurofins MWG Operon
Probe sequences Location and identity of any modifications Manufacturer of oligonucleotides Purification method qPCR protocol Complete reaction conditions	D D E D D E	Yes Yes Yes Yes Yes	Modification of the quenchers (BHQ1) Eurofins MWG Operon
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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), Mg2 , and dNTP concentrations Polymerase identity and concentration Buffer/kit identity and manufacturer Exact chemical composition of the buffer	D D E D D E E E E E E E	Yes Yes Yes Yes Yes Yes Yes Yes No	Modification of the quenchers (BHQ1) Eurofins MWG Operon Desalting (HPSF*) Bio-Rad (Hercules, CA), cat#186-3010 Not disclosed by the manufacter
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Number and concordance of biological replicates	D	Yes	cv analysis of the replicates
Number and stage (reverse transcription or qPCR) of technical replicates	E	Yes	
Repeatability (intraassay variation)	E	Yes	
Reproducibility (interassay variation, CV)	D	No	n.a. (done in only one laboratory and istrument)
Power analysis	D	No	
Statistical methods for results significance	E	Yes	Via cv analysis.
Software (source, version)	E	Yes	Excel spreadsheet
Cq or raw data submission with RDML	D	No	

Checklist from Bustin *et al.* Clinical Chemistry 55:4 611–622 (2009) showing (E) essential and (D) desirable information to be included in research reports using qPCR.

* Information not provided in this work but reference is made to original publication for the validation of the qPCR assays. http://gmo-crl.jrc.ec.europa.eu/summaries/Mon810_validation_report.pdf n.a. : not applicable to the study.