Quantitative PCR method for detection of cotton event MON 15985

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of cotton event 15985 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-GH-005

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM cotton event MON 15985 seeds

Tested GM events

Event Name	MON 15985
Unique Identifier	MON-15985-7
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.5% and 6.0% of cotton event MON 15985 DNA in non-GM cotton DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the acyl carrier protein 1 (*acp1*) reference gene and for the MON 15985 specific system. Four replicates for GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.05%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.085%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.10%	0.40%	0.90%	2.5%	6%
Mean Value (%)	0.08%	0.33%	0.84%	2.5%	6%
RSD_r (%)	19%	16%	22%	26%	15%
RSD_R (%)	42%	33%	27%	27%	16%
Bias %	-21%	-18%	-7.2%	-0.5%	0.5%

	GMO Target	Taxon Target
Mean Slope	-3.2	-3.3
Mean PCR Efficiency %	110	103
Mean R^2	0.99	0.98

Comment
The relative LOD and LOQ values were provided by the method developer and were not assessed in the collaborative trial.

3. REFERENCES

Savini C, Mazzara M, Munaro B, Van Den Eede G. Event-specific Method for the Quantification of Cotton Line MON 15985 Using Real-timePCR - Validation Report and Protocol. EUR 23650 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48908 (ISBN 978-92-79-11050-4)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTTACTAGATCGGGGATATCC-3'
Target element	Insert
Primer Reverse	5'-AAGGTTGCTAAATGGATGGGA-3'
Target element	3'-host genome
Amplicon length	82 bp
Probe	5'-FAM-CCGCTCTAGAACTAGTGGATCTGCACTGAA-TAMRA-3'
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-ATTGTGATGGGACTTGAGGAAGA-3'
Target element	*acp1*
Primer Reverse	5'-CTTGAACAGTTGTGATGGATTGTG-3'
Target element	*acp1*
Amplicon length	76 bp
Probe	5'-FAM-ATTGTCCTCTTCCACCGTGATTCCGAA-TAMRA-3'
Target element	acyl carrier protein 1 (*acp1*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	**Temperature**	**Time**	**No Cycles**
Decontamination (UNG)	50°C	120"	1
Activation/Initial Denaturation	95°C	600"	1
Denaturation	95°C	15"	
Annealing & Extension	60°C	60"	
Denaturing, Annealing & Extension			45