Cotton quantitative PCR methods

Quantitative PCR method for detection of cotton event GHB 614 (QT-EVE-GH-006)

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of cotton event GHB 614 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-GH-006

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from non-GM and GM cotton event GHB 614 cotton

Tested GM events

Event Name	GHB 614
Unique Identifier	BCS-GH002-5
Crop Name	*Gossypium hirsutum* L.

Collaborative Trial Description

The participants received twenty unknown samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 2% and 4.5% of cotton event GHB614 DNA in non-GM cotton DNA. In addition participants received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the alcohol dehydrogenase C (*adhC)* reference gene and for the GHB 614 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.023%	**LOD Absolute**	not reported
LOQ Relative	≤ 0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	0.09%	0.40%	0.90%	2.0%	4.5%
Mean Value (%)	0.10%	0.46%	0.97%	2.2%	4.6%
RSD_r (%)	9.4%	15%	6.8%	3.3%	4.1%
RSD_R (%)	12%	17%	8.3%	4.4%	5.1%
Bias %	15%	14%	7.5%	8.8%	2.0%

	GMO Target	Taxon Target
Mean Slope	-3.5	-3.5
Mean PCR Efficiency %	94	92
Mean R^2	1.00	1.00

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

3. REFERENCES

Savini C, Bogni A, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Cotton Line GHB 614 Using Real-time PCR - Validation Report and Protocol. EUR 23648 EN. Luxembourg (Luxembourg): OPOCE; 2008. JRC48918 *(ISBN 978-92-79-11048-1)*

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-CAAATACACTTGGAACGACTTCGT-3'
Target element	Insert
Primer Reverse	5'-GCAGGCATGCAAGCTTTTAAA-3'
Target element	3'-host genome
Amplicon length	120 bp
Probe	5'-FAM-CTCCATGGCGATCGCTACGTTCTAGAATT-TAMRA-3'
Probe Name	TM072
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-CACATGACTTAGCCCATCTTTGC-3'
Target element	*adhC*
Primer Reverse	5'-CCCACCCTTTTTTGGTTTAGC-3'
Target element	*adhC*
Amplicon length	73 bp
Probe	5'-VIC-TGCAGGTTTTGGTGCCACTGTGAATG-TAMRA-3'
Probe Name	TM012
Target element	alcohol dehydrogenase C (*adhC*) 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	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 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