

## Quantitative PCR method for detection of cotton event LLCotton25

### 1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of cotton event LLCotton25 and the cotton host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-GH-002

### 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 LLCotton25
Tested GM events	
Event Name	LLCotton25
Unique Identifier	ACS-GH001-3
Crop Name	<i>Gossypium hirsutum</i> L.

#### Collaborative Trial Description

The participants received twenty blind DNA samples representing five GM levels, namely 0.15%, 0.4%, 0.9%, 2.0% and 3.3% of cotton event LLCotton25 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 alcohol dehydrogenase C (*adhC*) reference gene and for the LLCotton25 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The  $\Delta C_t$  method was followed to calculate the GM content of the blind samples.

#### Method Performance

LOD Relative	≤ 0.045%	LOD Absolute	not reported
LOQ Relative	≤ 0.09%	LOQ Absolute	not reported

Values determined in the collaborative trial

<b>Test Level (%)</b>	<b>0.15%</b>	<b>0.40%</b>	<b>0.9%</b>	<b>2.0%</b>	<b>3.3%</b>
<b>Mean Value (%)</b>	0.17%	0.47%	1.1%	2.2%	3.6%
<b>RSD<sub>r</sub> (%)</b>	23%	28%	18%	18%	24%
<b>RSD<sub>R</sub> (%)</b>	23%	32%	32%	24%	30%
<b>Bias %</b>	12%	17%	20%	11%	8.1%

	<b>GM0 Target</b>
<b>Mean Slope</b>	-3.5
<b>Mean PCR Efficiency %</b>	91
<b>Mean R<sup>2</sup></b>	0.97

#### Comment

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

### 3. REFERENCES

Mazzara M, Grazioli E, Van Den Eede G. Event-Specific Method for the Quantification of Cotton Line “LLCotton25” Using Real-Time PCR - Validation Report and Protocol - Cotton Seeds Sampling and DNA Extraction. EUR 22912 EN. Luxembourg (Luxembourg): OPOCE; 2007. JRC37488 (ISBN 978-92-79-06932-1)

### 4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

<b>Primer Forward</b>	5'-CAAGGAACTATTCAACTGAG-3'
<b>Target element</b>	5'-host genome
<b>Primer Reverse</b>	5'-CAGATTTTGTGGGATTGGAATTC-3'
<b>Target element</b>	Insert
<b>Amplicon length</b>	79 bp
<b>Probe</b>	5'-FAM-CTTAACAGTACTCGGCCGTCGACGC-TAMRA-3'
<b>Probe Name</b>	TM018
<b>Target element</b>	DNA sequence in the 5' IBR

Taxon-target[s]

<b>Primer Forward</b>	5'-CACATGACTTAGCCCATCTTGC-3'
<b>Target element</b>	<i>adhC</i>
<b>Primer Reverse</b>	5'-CCCACCCTTTTTGGTTTAGC-3'
<b>Target element</b>	<i>adhC</i>
<b>Amplicon length</b>	73 bp
<b>Probe</b>	5'-FAM-TGCAGGTTTTGGTGCCACTGTGAATG-TAMRA-3'
<b>Probe Name</b>	TM012
<b>Target element</b>	alcohol dehydrogenase C ( <i>adhC</i> ) 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