

Quantitative PCR method for detection of soybean event A5547-127

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of soybean event A5547-127 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-GM-007

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 soybean event A5547-127 leaves
Tested GM events	
Event name	A5547-127
Unique Identifier	ACS-GM006-4
Crop Name	<i>Glycine max</i> L.

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.08%, 0.4%, 0.9%, 4.0% and 8.0% of soybean event A5547-127 DNA in non-GM soybean DNA. In addition the laboratories received five calibration samples, an amplification reagent control, reaction reagents, primers and probes for the lectin (*Le1*) reference gene and for the A5547-127 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 (%)	0.08%	0.40%	0.90%	4.0%	8.0%
Mean Value (%)	0.1%	0.44%	0.98%	4.1%	7.7%
RSD_r (%)	8%	7%	10%	5%	6%
RSD_R (%)	16%	16%	11%	9%	10%
Bias %	25%	10%	9%	2%	-4%

	GM0 Target	Taxon Target
Mean Slope	-3.5	-3.5
Mean PCR Efficiency %	93	94
Mean R²	1.00	1.00

Comment

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

3. REFERENCES

Charles Delobel C, Bogni A, Mazzara M, Van Den Eede G. Event-specific Method for the Quantification of Soybean Line A5547-127 Using Real-time PCR - Validation Report and Protocol. EUR 24240 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2009. JRC56620 (ISBN 978-92-79-14986-3)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCTATTTGGTGGCATTTCCTCA-3'
Target element	5'-host genome
Primer Reverse	5'-CACTGCGGCCAACTTACTTCT-3'
Target element	Insert
Amplicon length	75 bp
Probe	5'-FAM-CCGCAATGTCATACCGTCATCGTTGT-TAMRA-3'
Probe Name	TM058
Target element	DNA sequence in the 5' IBR

Taxon-target(s)

Primer Forward	5'-CTTTCTCGCACCAATTGACA-3'
Target element	<i>Le1</i>
Primer Reverse	5'-TCAAACCTCAACAGCGACGAC-3'
Target element	<i>Le1</i>
Amplicon length	102 bp
Probe	5'-VIC-CCACAAACACATGCAGGTTATCTTGG-TAMRA-3'
Probe Name	TM021
Target element	lectin (<i>Le1</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