GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Qualitative PCR method for detection of T35S pCAMBIA sequences (Rischitor et al., 2016)

Element specific

Last updated 21/12/2016

1. GENERAL INFORMATION

Target genetic element	Cauliflower Mosaic Virus 35S terminator (CaMV T-35S) in pCAMBIA vector
PCR Assay	Single
Detection	SybrGreen
Compendium Reference	QL-ELE-00-023

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 Bt rice grains (Breitler et al. 2004. Plant Biotechnology Journal 2: 417-430

Tested GM Events

Event Name	Unique Identifier	Crop Name
Bt rice	Not applicable	Oryza sativa

Collaborative Trial Description

The participants received 40 blinded test samples representing four GM levels containing 0, 5, 10 and 20 genomic copies of Bt rice DNA in non-GM rice DNA. In addition the laboratories received control samples, reaction reagents and primers for the 35S terminator specific system. Each GM level was tested in duplicate (20 measurements per level per lab) in one run. The participants scored the samples as positive when both the Cq value was lower than 40 for the fluorescence signal and the melting temperature was +/- 1.2 °C of the reference melting temperature (72.5 °C).

Method Performance

POD95	4.94 HGE	**Confidence Interval**	4.71-6.55 HGE

Values determined in the collaborative trial

False positive rate (%)	3.21
False negative rate (%)	2.39

	Test Level			
	0	5	10	20
POD (%)	2.92	96.07	98.92	97.86
CI upper	5.99	97.79	99.23	99.01
CI lower	1.7	93.1	95.89	95.4

Unit of Measurement Test Level: Genome copy N.

3. REFERENCES

Rischitor P, Lievens A, Mazzara M. Element-specific method for t35S-pCAMBIA detection using real-time PCR. Validation Report Qualitative Screening Method. 2016. JRC104515. Online Publication

4. PRIMERS AND PROBES SEQUENCES

GM-target(s) Cauliflower Mosaic Virus 35S terminator (CaMV T-35S) in pCAMBIA vector

Primer Forward	5'-CGGGGGATCTGGATTTTAGTA-3'
Target element	T35S pCAMBIA
Primer Reverse	5'-AGGGTTCCTATAGGGTTTCGCTC-3'
Target element	T35S pCAMBIA
Amplicon length	137 bp

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	**Final Concentration**
SYBR®Green PCR Master Mix 2x (Diagenode)	1x
Primer Fw	0.25 µmol/L
Primer Rev	0.25 µmol/L
Nuclease-free water	#
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	NoCycles
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			40
Melting point analysis	60°C-95°C	1200"	1

The melting curve analysis gradually increased the temperature from 60°C to 95°C in 20 min (*/-0.6°C/20s). Larger-than-forseen variation in melting temperature was observed during the initial analysis which resulted in false negatives. Each laboratory should estimate the Tm variation in its set-up and derive a Tm acceptance range accordingly. Any peak at the reference Tm should be considered sign of specific amplification, with the exception of peaks that have a height of less than 1/5th of the reference melting peak.