Potato quantitative PCR methods

Quantitative PCR method for detection of potato event EH92-527-1

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of potato event EH92-527-1 and the host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-ST-001

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 potato event EH92-527-1
Tested GM events	
Event Name	EH92-527-1
Unique Identifier	BPS-25271-9
Crop Name	*Solanum tuberosum* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.2% and 5.5% of potato event EH92-527-1 DNA in non-GM potato DNA. In addition the laboratories received four calibration samples, an amplification reagent control, reaction reagents, primers and probes for the UDP-glucose pyrophosphorylase *(UGPase)* reference gene and for the EH92-527-1 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	not reported	**LOD Absolute**	0.625
LOQ Relative	0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

Test Level (%)	**0.10%**	**0.40%**	**0.90%**	**2.2%**	**5.5%**
Mean Value (%)	0.11%	0.42%	0.97%	2.3%	5.7%
RSD_r (%)	12%	12%	10%	10%	10%
RSD_R (%)	16%	14%	13%	15%	12%
Bias %	4.9%	5.1%	8.2%	4.2%	4.3%

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.3
Mean PCR Efficiency %	96	95
Mean R^2	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

Savini C, Foti N, Mazzara M, Charles Delobel C, Van Den Eede G. Event-specific Method for the Quantification of Event EH92-527-1 Potato Using Real-time PCR - Validation Report and Protocol - Sampling and DNA Extraction of Potato. EUR 22358 EN. 2006. JRC34758 (ISBN 92-79-02988-6)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GTGTCAAAACACAATTTACAGCA-3'
Target element	Insert
Primer Reverse	5'-TCCCTTAATTCTCCGCTCATGA-3'
Target element	3'-host genome
Amplicon length	134 bp
Probe	5'-FAM-AGATTGTCGTTTCCCGCCTTCAGTT-TAMRA-3'
Probe Name	St527-S2
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-GGACATGTGAAGAGACGGAGC-3'
Target element	UGPase
Primer Reverse	5'-CCTACCTCTACCCCTCCGC-3'
Target element	UGPase
Amplicon length	88 bp
Probe	5'-FAM-CTACCACCATTACCTCGCACCTCCTCA-TAMRA-3'
Probe Name	UGP-sf1 probe
Target element	UDP-glucose pyrophosphorylase (*UGPase*) gene

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
PCR buffer II (10x)	1x	PCR buffer II (10x)	1x
ROX™ reference dye	1x	ROX™ reference dye	1x
Tween-20	0,01%	Tween-20	0,01%
Glycerol	0,8%	Glycerol	0,8%
dNTPs (dATP, dCTP, dGTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP)	200 µmol/L each
dUTP	400 µmol/L	dUTP	400 µmol/L
$MgCl_2$	4 mmol/L	$MgCl_2$	5,5 mmol/L
Primer Fw	0,30 µmol/L	Primer Fw	0,40 µmol/L
Primer Rev	0,30 µmol/L	Primer Rev	0,40 µmol/L
Probe	0,16 µmol/L	Probe	0,20 µmol/L
AmpliTaq Gold® DNA Polymerase	0,04 U/µL	AmpliTaq Gold® DNA Polymerase	0,04 U/µL
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
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45