GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of maize event Bt11 (Charles Delobel et al., 2008)

Event specific ***Maize***

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event Bt11 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan
Compendium Reference	QT-EVE-ZM-015

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 maize event Bt11 seeds

Tested GM Events

Event Name	Unique Identifier	Crop Name
Bt11	SYN-BT011-1	Zea mays

Collaborative Trial Description

The participants received 20 blind test samples representing five GM levels, namely 0.09%, 0.4%, 0.9%, 5.0% and 8.0% of maize event Bt11 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, reaction reagents, primers and probes for the maize alcohol dehydrogenase1 (*adh1*) reference gene and for the event Bt11 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The normalised DCt values of the unknown samples were measured and, by means of the regression formula of the standard curve, used to estimate the relative amount of event Bt11 DNA.

Method Performance

LOD Relative	≤0.04%	**LOD Absolute**	not reported
LOQ Relative	≤0.08%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

	Test Level (%)				
	0.09	0.4	0.9	5	8
Mean Value (%)	0.09	0.39	0.92	4.7	7.9
RSDr (%)	17%	13%	11%	13%	9%
RSDR (%)	24%	16%	15%	15%	14%
Bias (%)	2.2%	-1.9%	1.8%	-5.2%	-1.2%
Unit of Measurement Test Level	% GM copy N./reference genome copy N.				

	DCt
Mean Slope	-3.5
Mean PCR Efficiency %	93
Mean R2	0.99

Comment

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

3. REFERENCES

Charles Delobel C, Larcher S, Mazzara M, Van den Eede G. Event-specific Method for the Quantification of Maize Event Bt11 Using Real-time PCR - Validation Report and Protocol. EUR 23649 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2008. JRC48909 (ISBN 978-92-79-11049-8)

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4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of maize event Bt11 and the maize host genome
Primer Forward	5'-TGTGTGGCCATTTATCATCGA-3'
Target element	5'-host genome
Primer Reverse	5'-CGCTCAGTGGAACGAAAACTC-3'
Target element	insert
Amplicon lenght	68 bp
Probe	5'-FAM-TTCCATGACCAAAATCCCTTAACGTGAGT-TAMRA-3'

Taxon-target(s)	alcohol dehydrogenase1 (adh1) gene
Primer Forward	5'-CGTCGTTTCCCATCTCTTCCTCC-3'
Target element	adh1
Primer Reverse	5'-CCACTCCGAGACCCTCAGTC-3'
Target element	adh1
Amplicon lenght	135 bp
Probe	5'-VIC-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'

5. PCR REACTIONS SETUP

GM-target(s) **Taxon-target(s)**

Reagent	Final Concentration	Reagent	Final Concentration
Suppl. JumpStart Taq ReadyMix (2x) Sigma*	1x	Suppl. JumpStart Taq ReadyMix (2x) Sigma*	1x
Primer Fw	0.20 µmol/L	Primer Fw	0.30 µmol/L
Primer Rev	0.20 µmol/L	Primer Rev	0.30 µmol/L
Probe	0.15 µmol/L	Probe	0.20 µmol/L
Sulforhodamine 101	0.30 µmol/L	Sulforhodamine 101	0.30 µmol/L
$MgCl_2$	5.5 mmol/L	$MgCl_2$	5.5 mmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum	Template DNA	maximum
Final Volume	25 µL	Final Volume	25 µL

Comment *To prepare the supplemented JumpStart Taq ReadyMix (2x) Sigma solution Sulforhodamine 101and $MgCl_2$ were previously added in the appropriate concentration to the JumpStart TaqReadyMix (2x) from Sigma.

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-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