GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of maize event Bt11

Event specific ***Maize***

Last updated 15/12/2016

1. GENERAL INFORMATION

Target genetic element	3' 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-006

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 event Bt11 sweet maize

Tested GM Events

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

Collaborative Trial Description

The participants received twelve blind samples (six pairs of blind duplicate DNA samples) representing six GM levels, namely 0.1%, 0.3%, 0.7% 1.0%, 1.3% and 2% of Bt11 sweet maize DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, negative target controls consisting of non-GM maize DNA and Bt176 maize DNA extracted from Certified Reference Material, primers and probes for the alcohol dehydrogenase (*adh1*) reference gene and for the Bt11 specific system. Two replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	not reported
LOQ Relative	≤0.1%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

	Test Level (%)					
	0.1	0.3	0.7	1	1.3	2
Mean Value (%)	0.1	0.3	0.7	1	1.2	1.8
RSDr (%)	34%	19%	24%	10%	25%	15%
RSDR (%)	34%	19%	24%	13%	27%	18%
Bias (%)	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

Unit of Measurement Test Level % GM copy N./genome copy N.

	GMO Target	Taxon Target
Mean Slope	not reported	not reported
Mean PCR Efficiency %	not reported	not reported
Mean R2	not reported	not reported

Comment

The relative LOD and LOQ values were not assessed in the collaborative trial.

3. REFERENCES

Mazzara M, Puumalaainen J, Van Den Eede G. Validation of the GMO Specific Detection Method Developed by NVI/INRA for Bt11 in Sweet Corn Maize - Validation Report and Protocol. EUR 21829 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2005. JRC32111 (ISBN 92-79-00110-8)

ISO 21570:2005: Foodstuffs--Methods of Analysis for the Detection of Genetically Modified Organisms and Derived Products--Quantitative Nucleic Acid Based Methods. International Organisation for Standardisation, Geneva

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	3' integration border region (IBR) between the insert of maize event Bt11 and the maize host genome
Primer Forward	5'-GCGGAACCCCTATTTGTTTA-3'
Target element	insert
Primer Reverse	5'-TCCAAGAATCCCTCCATGAG-3'
Target element	3' junction
Amplicon length	70 bp
Probe	5'-FAM-AAATACATTCAAATATGTATCCGCTCA-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 length	135 bp
Probe	5'-FAM-AATCAGGGCTCATTTTCTCGCTCCTCA-TAMRA-3'

5. PCR REACTIONS SETUP

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

Reagent	Final Concentration	Reagent	Final Concentration
TaqMan Universal PCR Master Mix (2x)	1x	TaqMan Universal PCR Master Mix (2x)	1x
Primer Fw	0.75 µmol/L	Primer Fw	0.30 µmol/L
Primer Rev	0.75 µmol/L	Primer Rev	0.30 µmol/L
Probe	0.25 µmol/L	Probe	0.20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	max 200 ng	Template DNA	max 250 ng
Final Volume	25 µL	Final Volume	25 µL

Comment

The TaqMan buffer A (Life Technologies) originally used in the master mix for the quantification of event Bt11 has been discontinued by the manufacturer. The EURL GMFF suggests substituting it with the TaqMan Universal Master MIx (2x) reagent which has been successfully used in the master mix of other event-specific validated methods.

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			50