GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of maize event MIR604 (Mazzara et al., 2007)

Event specific ***Maize***

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of maize event MIR604 and the maize host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan
Compendium Reference	QT-EVE-ZM-013

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples from non-GM and GM maize event MIR604

Tested GM Events

Event Name	Unique Identifier	Crop Name
MIR604	SYN-IR604-5	Zea mays

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 2.5% and 6.0% of maize event MIR604 DNA in non-GM maize DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the alcohol dehydrogenase1 (*adh1)* reference gene and for the MIR604 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

Method Performance

LOD Relative	<0.045%	**LOD Absolute**	not reported
LOQ Relative	<0.09%	**LOQ Absolute**	not reported

Values determined in the collaborative trial

	Test Level (%)				
	0.1	0.4	0.9	2.5	6
Mean Value (%)	0.1	0.41	0.89	2.5	5.8
RSDr (%)	24%	17%	12%	16%	14%
RSDR (%)	27%	18%	18%	22%	20%
Bias (%)	3.6%	3.1%	-1.0%	0.7%	-3.6%
Unit of Measurement Test Level	% GMO copy N./genome copy N.				

	DCt
Mean Slope	-3.3
Mean PCR Efficiency %	97
Mean R2	1.00

Comment

The data on the LOD and LOQ was provided by the applicant and not assessed in the collaborative study.
In specificity testing, a relatively weak amplification with Ct values of 39.76 and 39.04 was reported with 100% GA21 DNA at 250 ng and 500 ng per reaction.

3. REFERENCES

Mazzara M, Munaro B, Foti N, Savini C, Van den Eede G. Event-specific Method for the Quantification of Maize Line MIR604 Using Real-time PCR - Validation Report and Protocol - Maize Seeds Sampling and DNA Extraction. EUR 22913 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2007. JRC37490 (ISBN 978-92 -79-06930-7)

DOI DOI: 10.2788/30596

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of maize event MIR604 and the maize host genome
Primer Forward	5'-GCGCACGCAATTCAACAG-3'
Target element	5'-host genome
Primer Reverse	5'-GGTCATAACGTGACTCCCTTAATTCT-3'
Target element	insert
Amplicon lenght	76 bp
Probe	5'-FAM-AGGCGGGAAACGACAATCTGATCATG-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	0.60 µmol/L	Primer Fw	0.30 µmol/L
Primer Rev	0.30 µmol/L	Primer Rev	0.30 µmol/L
Probe	0.20 µmol/L	Probe	0.20 µmol/L
Sulforhodamine 101	0.15 µmol/L	Sulforhodamine 101	0.15 µmol/L
$MgCl_2$	5.5 mmol/L	$MgCl_2$	5.5 mmol/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 supplemented JumpStart Taq ReadyMix (2x) Sigma solution was prepared by adding Sulforhodamine 101 and $MgCl_2$ in the appropriate concentration to the JumpStart TaqReadyMix (2x) from Sigma. For detection of stack events the method is still valid with a Sulforhodamine 101 final concentration of 0.30 µmol/L.

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