GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Qualitative PCR method for detection of nopaline synthase terminator

Element specific

Last updated 24/5/2017

1. GENERAL INFORMATION

Target genetic element	Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Simplex Real Time
Detection	TaqMan
Compendium Reference	QL-ELE-00-011

2. VALIDATION DATA

Collaborative trial coordinator	Federal Office of Consumer Protection and Food Safety (BVL)
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	CRM BF-415b and BF-415c (JRC-IRMM)

Tested GM Events

Event Name	Unique Identifier	Crop Name
NK603	MON-00603-6	Zea mays

Collaborative Trial Description

Each participant received 18 blind DNA samples of which 12 were prepared from GMO CRM (0.1% or 0.5% maize event NK603) and six from non-GM maize flour. In addition, participants received a positive DNA target control consisting of a solution of 0.5% mass fraction event NK603 DNA and solutions of primers, probe and a commercial reagent kit specific for the laboratory PCR platform. 12 laboratories used real-time PCR equipment designed for plastic reaction vials while other 12 laboratories equipped with a LightCycler instrument used glass capillaries. The participants were requested to submit the cycle threshold (Ct) values obtained in the T-nos real-time PCR with each sample and to report whether the sample was considered positive or negative.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	8 target copies

Values determined in the collaborative trial

False positive (%)	2.2%
False negative (%)	0%

	Test Level (%)		
	0	0.1	0.5
Specificity (%)	98%		
Sensitivity (%)		100%	100%

Unit of Measurement Test Level % GMO (w/w)

Comment

The performance values reported in the table for the 0.1% and 0.5% (w/w) levels were obtained with PCR equipment designed for plastic reaction vials. The false positives rate instead combines the data from both real-time PCR thermocyclers. The method was also positively tested in-house on samples of other T-nos containing GM-events such as soybean GTS-40-3-2, maize Bt11, NK603, GA21, MON809, MON863, CBH-351, MIR604, papaya SunUp, tomato Zeneca, canola MS1/RF1, MS8/RF3, and rice Oxy235 and BT63.

3. REFERENCES

Reiting R, Broll H, Waiblinger H-U, Grohmann L. Collaborative Study of a T-nos Real-Time PCR Method for Screening of Genetically Modified Organisms in Food Products. J. Verbr. Lebensm. 2007; 2: 116-121

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ISO 21569:2005/Amd 1:2013: Foodstuffs--Methods of analysis for the detection of genetically modified organisms and derived products -- Qualitative nucleic acid based methods Amendment 1

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	Nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
Primer Forward	5'-CATGTAATGCATGACGTTATTTATG-3'
Target element	T-nos
Primer Reverse	5'-TTGTTTTCTATCGCGTATTAAATGT-3'
Target element	T-nos
Amplicon length	84 bp
Probe	5'-FAM-ATGGGTTTTTATGATTAGAGTCCCGCAA-TAMRA-3'

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
QuantiTect Probe PCR Kit (Qiagen) 2x	1x
Primer Fw	0.40 µmol/L
Primer Rev	0.40 µmol/L
Probe	0.10 µmol/L
Template DNA	100 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	NoCycles
Activation/Initial Denaturation	95°C	900”	1
Denaturation	94°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			45