Qualitative PCR method for detection of nopaline synthase terminator (Barbau-Piednoir et al., 2014)

1. GENERAL INFORMATION

Target genetic element	Nopaline synthase terminator (T-nos) from Agrobacterium tumefaciens
PCR Assay	Simplex Real Time
Detection Chemistry	SybrGreen
Compendium Reference	QL-ELE-00-018

2. VALIDATION DATA

Collaborative trial coordinator	Scientific Institute of Public Health
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Plasmids "Sybricons" mix DNA solution (BCCM™/LMBP, Gent-Belgium)

Tested GM Events

Event Name	Unique Identifier	Crop Name
Bt11	SYN-BT011-1	Zea mays L.
GTS 40-3-2	MON-04032-6	Glycine max L.
RF3	ACS-BN003-6	Brassica napus L.
MON810	MON-00810-6	Zea mays L.
GT73 (RT73)	MON-00073-7	*Brassica napus L.*

Collaborative Trial Description

The participants received 10 blind DNA samples representing three controls (maize, soybean or oilseed rape DNA solutions) and seven GM levels, namely 0,1% GTS 40-3-2 soybean in 100% soybean; 2% Bt11 maize in 100% maize; 0,5% MON810 maize in 100% maize; 0,1% GT73 oilseed rape in 100% oilseed rape; 0,9% RF3 oilseed rape in 100% oilseed rape; 2% RF3 oilseed rape and 1% GTS 40-3-2 soybean in 100% maize; 0.1% GT73 oilseed rape and 0,5% MON810 maize in 40% oilseed rape and 60% maize. In addition the laboratories received the CoSYPS inter-laboratory protocol, the Excel interpretation matrix files (DSS), the Deviation Report Form, a DNA mix solution of plasmids "Sybricon" containing the amplicons produced by the corresponding qPCR methods as positive control, reaction reagents and primers and probes for performing the analyses. Each qPCR analysis was performed in six fold on each sample for the particular qPCR test. A qPCR test was considered "positive" when the observed C_t value was lower than 35 in combination with an acceptable T_m value (less than a 1C° difference related to the T_m value of the positive control). The outcome for each method was combined into a Gödel Prime number Product and interpreted using the prime-number GMO matrix table included in the CoSYPS DSS using a factorization algorithm.

Method Performance

LOD Relative	not reported	**LOD Absolute**	23 Tnos copies in 25 ng

Values determined in the collaborative trial

False positive (%)	3.5%
False negative (%)	1.1%

Test Level (%)	1	2	3	4	5	6	7	8
Specificity (%)					94%			99%
Sensitivity (%)		99%	99%	99%		100%	100%	

Unit of Measurement Test Level % w/w

Comment

Test samples 1 represented maize, soybean or oilseed rape control DNA solutions, while test samples 2, 3, 4, 5, 6, 7, 8 corresponded respectively to 0,1% GTS 40-3-2 soybean in 100% soybean; 2% Bt11 maize in 100% maize; 0,5% MON810 maize in 100% maize; 0,1% GT73 oilseed rape in 100% oilseed rape; 0,9% RF3 oilseed rape in 100% oilseed rape; 2% RF3 oilseed rape and 1% GTS 40-3-2 soybean in 100% maize; 0.1% GT73 oilseed rape and 0,5% MON810 maize in 40% oilseed rape and 60% maize

3. REFERENCES

E. Barbau-Piednoir, P. Stragier, N. Roosens, M. Mazzara, C. Savini, G. Van den Eede and M. Van den Bulcke (2014) "Inter-laboratory Testing of GMO Detection by Combinatory SYBR®Green PCR Screening (CoSYPS)". Food Anal. Methods. DOI 10.1007/s12161-014-9837-3

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4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	Nopaline synthase terminator (T-nos) from Agrobacterium tumefaciens
Primer Forward	5'-GATTAGAGTCCCGCAATTATACATTTAA-3'
Target element	T-nos
Primer Reverse	5'-TTATCCTAGKTTGCGCGCTATATTT-3'
Target element	T-nos
Amplicon lenght	69 bp

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
SYBR®Green qPCR Mastermix (Diagenode)	1x
Primer Fw	0,25 µmol/L
Primer Rev	0,25 µmol/L
Template DNA	50 ng
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	NoCycles
Activation/Initial Denaturation	95°C	600”	1
Denaturation	95°C	15”	
Annealing & Extension	60°C	60”	
Denaturing, Annealing & Extension			40
Melting point analysis	60°C-95°C	20 min	1