Qualitative PCR method for detection of Figwort Mosaic Virus 35S promoter

Target genetic element	Figworth Mosaic Virus 35S promoter (P-FMV)
PCR Assay	Single
Detection Chemistry	Agarose gel ectrophoresis
Compendium Reference	QL-ELE-00-010

2. VALIDATION DATA

Collaborative trial coordinator	GMO Detection Laboratory of Shanghai Entry-Exit Inspection & Quarantine Bureau
Test material applied in collaborative trial	Oilseed rape meal
Materials used for calibration/controls	Meal derived from dried grain samples of oilseed rape event GT73 (Monsanto)

Tested GM Events

Event Name	GT73 (RT73)
Unique Identifier	MON-00073-7
Crop Name	*Brassica napus L.*

Collaborative Trial Description

Each laboratory received 20 encoded dried meal samples, including 10 samples for the detection of the oilseed rape high mobility group protein I/Y (HMGa) endogene and 10 samples for the detection of the FMV 35S promoter. The first series of samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) non-GM oilseed rape in a rice background. The second series of samples contained duplicate 5%, 1%, 0.1%, 0.05%, and 0.01% (w/w) GT73 oilseed rape in non-GM oilseed rape samples. The participants were asked to extract DNA from the samples according to the protocol provided and to perform the corresponding PCR detection assays. The amplified PCR products were analyzed by gel electrophoresis. The detection of a DNA fragment with the same size as the positive control indicated that the sample was positive; otherwise the result was considered negative.

Method Performance

LOD Relative	≤0.1%	**LOD Absolute**	75 HGE

Values determined in the collaborative trial

False positive (%)	not reported
False negative (%)	1.4%

Test Level (%)	0	0.01	0.05	0.1	1	5
Specificity (%)						
Sensitivity (%)		21%	71%	96%	100%	100%

Comment

The value of false negatives is calculated on the samples with a concentration of analyte above the LOD level

3. REFERENCES

L. Pan, S. Zhang, L. Yang, H. Broll, F. Tian, and D. Zhang (2007) “Interlaboratory Trial Validation of an Event-Specific Qualitative Polymerase Chain Reaction-Based Detection Method for Genetically Modified RT73 Rapeseed” Journal of AOAC International, Vol. 90, No. 6, p. 1639-1646

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	Figworth Mosaic Virus 35S promoter (P-FMV)
Primer Forward	5'-AAGCCTCAACAAGGTCAG-3'
Target element	P-FMV
Primer Reverse	5'-CTGCTCGATGTTGACAAG-3'
Target element	P-FMV
Amplicon lenght	196 bp

Taxon-target(s)	high mobility group protein I/Y (HMGa) gene
Primer Forward	5'-GGTCGTCCTCCTAAGGCGAAAG-3'
Target element	HMGa
Primer Reverse	5'-GCAACCAACAGGCACCATC-3'
Target element	HMGa
Amplicon lenght	219 bp

5. PCR REACTIONS SETUP

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

Reagent	Final Concentration	Reagent	Final Concentration
Taq DNA Polymerase buffer 10x (Roche)	1x	Taq DNA Polymerase buffer 10x (Roche)	1x
$MgCl_2$	1,5 mmol/L	$MgCl_2$	1,5 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each	dNTPs (dATP, dCTP, dGTP, dTTP)	200 µmol/L each
Primer Fw	0,20 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,20 µmol/L	Primer Rev	0,20 µmol/L
Taq DNA Polymerase	1,0 U	Taq DNA Polymerase	1,0 U
Template DNA	10-50 ng	Template DNA	10-50 ng
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s) and taxon-target(s)

Stage	Temperature	Time	NoCycles
Activation/Initial Denaturation	94°C	180”	1
Denaturation	94°C	30”	
Annealing	54°C	30”	
Extension	72°C	40”	
Denaturing, Annealing & Extension			40
Final Extension	72°C	180”	1