Qualitative duplex PCR method for detection of Cauliflower Mosaic Virus 35S promoter and nopaline synthase terminator (partim CaMV P-35S)

1. GENERAL INFORMATION

Target genetic elements	Cauliflower Mosaic Virus 35S promoter (CaMV P-35S) and nopaline synthase terminator (T-nos) from *Agrobacterium tumefaciens*
PCR Assay	Duplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QL-ELE-00-012

2. VALIDATION DATA

Collaborative trial coordinator	Chemisches und Veterinaruntersuchungsamt Freiburg
Test material applied in collaborative trial	Genomic DNA
Materials used for calibration/controls	CRM BF412F (JRC-IRMM)
Tested GM events	
Event Names	Bt11; MON810; GA21
Unique Identifier	SYN-BT011-1; MON-00810-6; MON-00021-9
Crop Name	*Zea mays* L.

Collaborative Trial Description

The inter-laboratory study was conducted to evaluate a duplex real-time PCR screening method for the simultaneous detection and semi-quantitative estimation of CaMV 35S promoter and T-nos sequences in transgenic maize reference samples. Participants received nine different maize DNA mixtures containing the transformation events Bt11, MON 810 and/or GA21 at different concentration levels (see endnote). In addition each laboratory received one negative GM maize DNA sample, DNA calibration standards extracted from Bt11 reference material for the quantification of the CaMV P-35S and T-nos sequence and reagents. Each DNA sample had to be analyzed in five replicas.

Method Performance

LOD Relative	≤0.02%	**LOD Absolute**	not reported
LOQ Relative	not reported	**LOQ Absolute**	not reported

Values determined in the collaborative trial (partim CaMV P-35S)

False positive (%)	18%
False negative (%)	not reported

Test Level	1	2	3	4	5	6	7	8	9
Sensitivity (%)	100%	100%	100%	100%	100%	-	-	100%	100%
Mean Value	13	56	470	1170	29			43	1192
RSD_r (%)	38%	27%	15%	10%	32%	-	-	42%	13%
RSD_R (%)	-	-	-	-	-	-	-	-	-

	GMO Target
Mean Slope	not reported
Mean PCR Efficiency %	93%
Mean R^2	not reported

Comment
The relative LOD was not determined in this collaborative trial.

3. REFERENCES

H.-U. Waiblinger, B. Ernst, A. Anderson, and K. Pietsch "Validation and collaborative study of a P35S and T-nos duplex real-time PCR screening method to detect genetically modiWed organisms in food products" Eur Food Res Technol (DOI 10.1007/s00217-007-0748-z)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-GCCTCTGCCGACAGTGGT-3' 5'-CATGTAATGCATGACGTTATTTATG-3'
Target element	*CaMV P-35S* *T-nos*
Primer Reverse	5'-AAGACGTGGTTGGAACGTCTTC-3' 5'-TTGTTTTCTATCGCGTATTAAATGT-3'
Target element	*CaMV P-35S* *T-nos*
Amplicon length	82 bp 84 bp
Probe	5'-FAM-CAAAGATGGACCCCCACCCACG-BHQ1-3' 5'-YY-ATGGGTTTTTATGATTAGAGTCCCGCAA-BHQ1-3'
GMO Target element	CaMV 35S promoter and nopaline synthase terminator

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
TaqMan® Universal PCR Master Mix	1x
Primer 35S-F	0,10 µmol/L
Primer 35S-R	0,10 µmol/L
Probe 35S-TMP FAM	0,10 µmol/L
Primer 180-F	1,0 µmol/L
Primer 180-R	1,0 µmol/L
Probe TM-180 YY	0,20 µmol/L
Template DNA	5 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	No Cycles
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			45

Endnote on the composition of the respective tested combinations:

0.02% Bt11 (CAMV P-35S+; T-nos+)(Level 1), 0.1% Bt11 (CAMV P-35S+; T-nos+) (Level 2), 1.0% Bt11 (CAMV P-35S+; T-nos+) (Level 3), 0.05% MON810 (CAMV P-35S+; T-nos-) (Level 4), 2.5% MON810 (CAMV P-35S+; T-nos-) (Level 5), 0.05% GA21 (CAMV P-35S-;T-nos+) (Level 6), 2.5% GA21 (CAMV P-35S-; T-nos+) (Level 7), 0.05% MON810 + 2.5% GA21 (CAMV P-35S+; T-nos+) (Level 8) and 2.5% MON810 + 0.05% GA21 (CAMV P-35S+; T-nos+) (Level 9)