QL-EVE-DC-006

Qualitative PCR method for detection of carnation event 123.8.8 (EURL GMFF, 2016)

1. General information

Specificity: Event specific
Analysis type: Qualitative
Species: Carnation
GMO: 123.8.8 (40685) (FLO-40685-2)
Genetic target: 123.8.8 insert
Technology: Endpoint PCR (Multiplex): QL-EVE-DC-006 + QL-TAX-DC-001
Comments:
Amplicon: TGGTGGACCCTTGAGGAAACTGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNCTACATCAGTCAGCCATTGTAT

Oligos:

Type	Name(s)	Sequence	Length (bp)	Target
Primer forward	RBF1a	TGGTGGACCCTTGAGGAAACTG	22	insert
Primer reverse	123.8.8-2.1R	ATACAATGGCTGACTGATGTAG	22	3'-host genome

2. Validation

2.1. General data

Method validated in combination: QL-TAX-DC-001

Type	Name(s)	Sequence	Length (bp)	Target
Primer forward	ANS.F	CTAGATCGGAGGTCACCATACC	22	ANS
Primer reverse	ANS.R	GAAACCGTGACCATGGTCTCG	21	ANS

Matrix type: DNA
Coordinator: JRC Directorate F - Health, Consumers and Reference Materials
Validation type CRL / InHouse
Analysis type: Qualitative
Purpose: Identification
Description: The EURL GMFF tested the method on genomic DNA samples of the conventional parental carnation line (negative control sample) and of the GM line 123.8.8 (positive control sample). Tests were performed in duplicate using a duplex PCR targeting the anthocyanidin synthase carnation gene (ANS) and the GM line 123.8.8. The amplified PCR products were separated by agarose gel electrophoresis and visualized under UV light. Their identity was confirmed by restriction digestion with the ClaI (591bp + 688 bp) and AgeI (95 bp + 243 bp) enzymes respectively. The robustness of the method was tested by changing the PCR reaction components. The limit of detection (LOD) was calculated by amplifying with the GM duplex system four different GM-levels having 2500, 1000, 250 or 25 GM genomes per reaction. Sixty replicates were tested for each dilution. The specificity of the event 123.8.8 detection method was verified on the carnation GM lines 123.2.38 (FLO-40644-6), 123.8.12 (FLO-040689-6), IFD-25958-3, IFD-26407-2 and SHD-27531-4.

2.2. Performance

	QL-EVE-DC-006 *	QL-TAX-DC-001
LOD absolute	> 250 HGE	
LOD relative	not reported	
False positive rate	0	
False negative rate	0	

* The method is not suitable for quantification, due to uncertainties on the copy number of the anthocyanidin synthase (ANS) reference gene.

Collaborative trial (QL-EVE-DC-006)

Unit of measurement: GM genomes copies

	0	25	250	1000	2500
Specificity	100%				
Sensitivity		0%	0%	100%	100%

2.2. Experimental details

QL-TAX-DC-001 / QL-EVE-DC-006

Detection chemistry	Agarose gel electrophoresis
CRM	Genomic DNA samples extracted from non-GM and GM carnation event 123.8.8

2.3. Method stages

QL-TAX-DC-001 / QL-EVE-DC-006

Stage	Cycles	Time	Temperature	Ramp rate
Activation/Initial Denaturation	1	600"	95°C	
Denaturation		30"	95°C	
Annealing		30"	55°C	
Extension		60"	72°C	
Denaturation, Annealing and Extension	27			
Final Extension	1	420"	72°C	

2.4. Method reagents

QL-TAX-DC-001 / QL-EVE-DC-006

Reagent	Final concentration
AmpliTaq Gold 360 Master Mix 2x (Applied Biosystems)	1x
Primer RBF1a Fw	0.40 umol/L
Primer 123.8.8-2.1R	0.40 umol/L
Primer ANS.F	0.40 umol/L
Primer ANS.R	0.40 umol/L
Template DNA	100 ng
Nuclease-free water	#
Final volume	25 uL

3. References

European Union Reference Laboratory for GM Food and Feed (EURL GMFF), Joint Research Centre (JRC), European Commission. Report on the Single-laboratory Validation of a PCR-based Detection Method for Identification of GM-line FLO-40685-2 Carnation - JRC Validated Methods,

Reference Methods and Measurement Reports. JRC103658. 2016. | 2016