European Commission

GMOMETHODS: EU database of reference methods

QL-EVE-DC-004

Qualitative PCR method for detection of carnation event 26407 (EURL GMFF, 2013)

1. General information

Specificity: Event specific
Analysis type: Qualitative
Species: Carnation
GMO: 26407 (IFD-26407-2)
Genetic target: 26407 insert
Technology: Endpoint PCR (Multiplex): QL-EVE-DC-004 + QL-TAX-DC-001
Comments:
Amplicon: ATTTCCACCTTCACCTACGATGGNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTCATCCAATTTGTACCCGTG

Oligos:

Type	Name(s)	Sequence	Length (bp)	Target
Primer forward	RB Forward	ATTTCCACCTTCACCTACGATGG	23	insert
Primer reverse	IFD-26407-2 Reverse	CACGGGTACAAATTGGATGAG	21	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-IHCP
Validation type CRL / InHouse
Analysis type: Qualitative
Purpose: Identification
Description: The EURL GMFF tested the method on genomic DNA samples of a conventional (negative control sample) carnation line and of the GM line 26407 (positive control sample). Tests were performed in duplicate with two different DNA amounts using a duplex PCR targeting the anthocyanin synthase carnation gene (ANS) and the GM line 26407. The amplified PCR products were analyzed by agarose gel electrophoresis and further confirmed by restriction digestion with the EcoRV enzyme. The robustness of the method was tested by performing the PCR reaction with a different Taq DNA polymerase and reaction buffer. The limit of detection (LOD) was calculated by amplifying with the GM duplex system a serial dilution of event 26407 genomic DNA having defined copy numbers per reaction. Sixty replicates were tested for each dilution.

2.2. Performance

	QL-EVE-DC-004 *	QL-TAX-DC-001
LOD absolute	<= 50 HE	
LOD relative	not reported	
False positive rate	0	
False negative rate	0	

* The robustness tests indicated that the proposed detection method tolerates changes in the brand of the Taq DNA polymerase and related reaction buffer.

Collaborative trial (QL-EVE-DC-004)

Unit of measurement: Target copy N.

	0	0.1	10	50
Specificity	100%			
Sensitivity		0%	70%	100%

2.2. Experimental details

QL-TAX-DC-001 / QL-EVE-DC-004

Detection chemistry	Agarose gel electrophoresis
Confirmation of amplification	Restriction digestion with EcoRV (105 bp+386 bp)
CRM	Genomic DNA samples extracted from leaf tissue of non-GM and GM carnation event 26407

2.3. Method stages

QL-TAX-DC-001 / QL-EVE-DC-004

Stage	Cycles	Time	Temperature	Ramp rate
Activation/Initial Denaturation	1	900"	95°C	
Denaturation		30"	95°C	
Annealing		30"	55°C	
Extension		60"	72°C	
Denaturation, Annealing and Extension	30			
Final Extension	1	300"	72°C	

2.4. Method reagents

QL-TAX-DC-001 / QL-EVE-DC-004

Reagent	Final concentration
DNA Polymerase buffer 10x (Qiagen)	1x
dNTPs (dATP, dCTP, dGTP, dTTP)	200 umol/L each
Primer RB forward	4.0 ng/uL
Primer IFD-26407-2 reverse	4.0 ng/uL
Primer ANS.F	4.0 ng/uL
Primer ANS.R	4.0 ng/uL
Template DNA	100 ng
HotStar Taq DNA Polymerase (Qiagen)	2.5 U
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 FlorigeneTM 26407 GM Carnation - Validation Report and Validated Method. Luxembourg (Luxembourg): Publications Office of the European Union. JRC85668. 2013. | 10.2788/34771 | 2013