

Event specific Soybean

Quantitative PCR method for detection of soybean event MON89788 (Charles Delobel et al., 2013)

1. GENERAL INFORMATION

Target genetic element	5' integration border region (IBR) between the insert of soybean event MON 89788 and the soybean host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan
Compendium Reference	QT-EVE-GM-006

2. VALIDATION DATA

Collaborative trial coordinator	JRC-IHCP
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA samples extracted from non-GM and GM soybean event MON 89788 seeds
Tested GM Events	
Event Name	MON89788
Unique Identifier	MON-89788-1
Crop Name	<i>Glycine max L.</i>

Collaborative Trial Description

The participants received 20 blind samples representing five GM levels, namely 0.1%, 0.4%, 0.9%, 4.0% and 8.0% of soybean event MON 89788 DNA in non-GM soybean DNA. In addition, the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the lectin (Le1) reference gene and for the MON 89788 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system.

Method Performance

LOD Relative	≤ 0.045%	LOD Absolute	not reported
LOQ Relative	≤0.09%	LOQ Absolute	not reported

Values determined in the collaborative trial

Test Level (%)	0.1	0.4	0.9	4	8
Mean Value (%)	0.09	0.38	0.89	4.4	8.2
RSDr (%)	16%	22%	15%	13%	12%
RSDR (%)	20%	25%	18%	16%	12%
Bias (%)	-14%	-5%	-0.9%	11%	2.8%

Unit of Measurement Test Level copy/copy

	GMO Target	Taxon Target
Mean Slope	-3.4	-3.3
Mean PCR Efficiency %	98	100
Mean R2	1.00	1.00

Comment

The LOD and LOQ values were provided by the method's developer and were not further assessed in the collaborative trial.

3. REFERENCES

Charles Delobel C, Bogni A, Pinski G, Mazzara M, Van den Eede G. Event-specific Method for the Quantification of Soybean Line MON 89788 Using Real-time PCR v 1.01 - Validation Report and Validated Method. EUR 26153 EN. Luxembourg (Luxembourg): Publications Office of the European Union; 2013. JRC84189 (ISBN 978-92-79-33078-0)

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4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	5' integration border region (IBR) between the insert of soybean event MON 89788 and the soybean host genome
Primer Forward	5'-TCCCGCTCTAGCGCTTCAAT-3'
Target element	5'-host genome
Primer Reverse	5'-TCGAGCAGGACCTGCAGAA-3'
Target element	insert
Amplicon length	139 bp
Probe	5'-FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA-3'

Taxon-target(s)	lectin (Le1) gene
Primer Forward	5'-CCAGCTTCGCCGCTTCCTTC-3'
Target element	Le1
Primer Reverse	5'-GAAGGCAAGCCCATCTGCAAGCC-3'
Target element	Le1
Amplicon length	74 bp
Probe	5'-FAM-CTTCACCTTCTATGCCCTGACAC-TAMRA-3'

5. PCR REACTIONS SETUP

GM-target(s)		Taxon-target(s)	
Reagent	Final Concentration	Reagent	Final Concentration
TaqMan Universal PCR Master Mix (2x)	1x	TaqMan Universal PCR Master Mix (2x)	1x
Primer Fw	0,15 µmol/L	Primer Fw	0,15 µmol/L
Primer Rev	0,15 µmol/L	Primer Rev	0,15 µmol/L
Probe	0,05 µmol/L	Probe	0,05 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	50 µL	Final Volume	50 µL

6. AMPLIFICATION CONDITIONS

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

Stage	Temperature	Time	NoCycles
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