GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Qualitative LAMP method for detection of *Cry1Ac* gene (Li et al., 2018)

Element specific

Last updated 15/12/2017

1. GENERAL INFORMATION

Target genetic element	Cry1Ac modified gene derived from Bacillus thuringiensis
Assay	LAMP
Detection	SybrGreen
Compendium Reference	QL-ELE-00-030

2. VALIDATION DATA

Collaborative trial coordinator	National Center of Molecular Characterisation for GMOs, Shanghai Jiao Tong University (NCMCG-SJTU)
Test material applied in collaborative trial	DNA
Materials used for calibration/controls	Genomic DNA extracted from GM and non-GM crop seeds or leaves

Tested GM Events

Event Name	Unique Identifier	Species
MON531	MON-00531-6	Gossypium hirsutum
MON15985	MON-15985-7	Gossypium hirsutum

Collaborative Trial Description

The participants received 4 DNA samples for performing specificity tests, a GM positive DNA stock solution at a concentration of 25 ng/µL for the sensitivity studies and LAMP primers and reagents for detecting the Cry1Ac gene. In addition the laboratories received 5 blind DNA samples, consisting of a blank control sample, a sample containing a mixture of non-GM maize, rice, soybean, cotton, wheat, and oilseed rape genomic DNA and three samples comprising each a mixture of two different GM events at 0.5% level, characterised for distinctive presence or absence of the Cry1Ac gene. For the sensitivity studies the participants were requested to prepare, according to a provided protocol, a dilution series containing 25, 10, 5, 2.5 and 1 HGE/uL of the cotton event MON15985 which is positive for the Cry1Ac gene. Each LAMP assay was repeated three times and each time with three replicates. The LAMP reactions products were examined by visual inspection after staining with 1 µL of SYBR Green I (1000x) fluorescent dye or by using a gel Image System after agarose gel electrophoresis and staining with Gel-red dyer.

Method Performance

LOD Relative	not reported	**LOD Absolute**	≤10 HGE

Values determined in the collaborative trial

False positive (%)	0%
False negative (%)	0%

	Test Level				
	1	2	3	4	5
Specificity (%)	100%	100%		100%	100%
Sensitivity (%)			100%		

Unit of Measurement Test Level % GMO copy N./genome copy N.

Comment

The five blind DNA samples were as follows: sample 1 contained 0.5% GM rice T1C-19 (Cry1Ac-) and 0.5% GM rice Bt63 (Cry1Ac-); sample 2 contained 0.5% GM maize Bt176 (Cry1Ac-) and 0.5% GM T25 (Cry1Ac-); sample 3 contained 0.5% GM cotton MON15985 (Cry1Ac+) and 0.5% GM cotton MON88913 (Cry1Ac-); sample 4 contained 0.5% GM oilseed rape RF1 (Cry1Ac-) and 0.5% GM oilseed rape GT73 (Cry1Ac-); sample 5 contained 0.5% GM soybean A5547-127 (Cry1Ac-)

3. REFERENCES

Li R, Shi J, Liu B, Zhang D, Zhao X, Yang L. International collaborative ring trial of four gene-specific loop-mediated isothermal amplification assays in GMO analysis. Food Control 2018; 84: 278-283

DOI 10.1016/j.

Wang C, Li R, Quan S, Shen P, Zhang D, Shi J, Yang L. GMO detection in food and feed through screening by visual loop-mediated isothermal amplification assays. Anal. and Bioanal. Chem. 2015; 407: 4829-4834

DOI 10.1007/s00216-015

4. PRIMERS AND PROBES SEQUENCES

GM-target(s) Cry1Ac modified gene derived from Bacillus thuringiensis

Primer Name	Primer sequence	Target element
cry1Ac F3	5'-GAGGCCAAAGAGTCCGTG-3'	Cry1Ac
cry1Ac FIP	5'-GGCGTGGATCATGGCGATGTTGCTTTGTTCGGGAACTCCCA-3'	Cry1Ac
cry1Ac loop F	5'-GTGTCGGCTTGCAACTGATCATA-3'	Cry1Ac
cry1Ac B3	5'-GCGGTAAAGATACGTCCCTC-3'	Cry1Ac
cry1Ac BIP	5'-ACGTGTGCACAGCATTCGTGAGTTCCTCGAAGATGGCAGC-3'	Cry1Ac
cry1Ac loop B	5'-CCTGAGTTGTCCGTGATCCCTG-3'	Cry1Ac
Amplicon length	197 bp	

5. PCR REACTIONS SETUP

GM-target(s)

Reagent	Final Concentration
Mg^{2+}	25 mmol/L
dNTPs (dATP, dCTP, dGTP, dTTP)	2 mmol/L
Bst DNA Polymerase L.F. (New England Biolabs)	8.0 U
Betaine (Sigma)	5.0 mol/L
Thermol buffer (10x)	1x
Primer F3	10.0 µmol/L
Primer B3	10.0 µmol/L
Primer FIP	10.0 µmol/L
Primer BIP	10.0 µmol/L
Primer Loop F	10.0 µmol/L
Primer Loop B	10.0 µmol/L
Template DNA	2.0 µL
Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

GM-target(s)

Stage	Temperature	Time	NoCycles
Annealing & Extension	63°C	60”	1
Deactivation	80°C	#	1
Cooling	4°C	#	1