GMOMETHODS: EU DATABASE OF REFERENCE METHODS

Quantitative PCR method for detection of rice event Golden Rice 2 (Jacchia et al., 2015)

Event specific ***Rice***

Last updated 8/4/2016

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of rice event Golden Rice 2 and the rice host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-OS-001

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 event Golden Rice 2 frozen leaves

Tested GM Events

Event Name	Unique Identifier	Crop Name
GR2	Not applicable	*Oryza sativa*

Collaborative Trial Description

The participants received two set of samples, one, labeled PLD, for the validation of the taxon-specific assay, and one, labeled GR2, for the validation of the GR2 event-specific assay and of the entire method. The two set of samples consisted each of five standard curve samples prepared by serial dilution's respectively of non-GM and GM DNA and 20 blind samples representing 5 levels. The PLD blind samples were prepared with non-GM DNA, diluted to obtain 200000, 20000, 2000, 200 and 50 HGE copies per reaction. GR2 blind DNA samples were prepared with GM DNA, diluted in a background of non-GM DNA to obtain 20000, 6000, 1800, 200 and 50 GR2 HGE copies per reaction corresponding to a GR2 GM % of 10%, 3%, 0.9%, 0.1% and 0.025 %. The participants also received all reagents, a detailed protocol and an excel file for reporting the results. The laboratories had to run three qPCR plates: one loaded with PLD standard curve samples and PLD blind samples (plate A) for the quantification of the PLD blind samples with the PLD taxon-specific assay; one loaded with GR2 standard curve samples and GR2 blind samples (plate B) for the quantification of the GR2 blind samples with the GR2 event-specific assay; and one loaded with the PLD standard curve samples and GR2 blind samples (plate C) for the quantification of the rice taxon-specific PLD target in GR2 blind samples with the PLD taxon-specific assay. The four blind samples with the same HGE content were considered as independent replicates.

Method Performance

LOD Relative	not reported	**LOD Absolute**	4 HGE
LOQ Relative	not reported	**LOQ Absolute**	40 HGE

Values determined in the collaborative trial

	Test Level (%)				
	0.025	0.1	0.9	3	10
Mean Value (%)	0.0296	0.12	1.05	3.3	10.4
RSDr (%)	12%	7.6%	5.6%	6.7%	7%
RSDR (%)	15%	12%	11%	12%	12%
Bias (%)	18%	16%	17%	10%	3.6%
Unit of Measurement Test Level	% GMO copy N./genome copy N.				

	GMO Target	Taxon Target
Mean Slope	-3.65	-3.43
Mean PCR Efficiency %	88	96
Mean R2	1.00	1.00

3. REFERENCES

Jacchia S, Nardini E, Bassani N, Savini C, Shim J-H, Trijatmiko K, Kreysa J, Mazzara M. International ring trial for the validation of an event-specific Golden Rice 2 quantitative real-time polymerase chain reaction method. J. Agric. Food Chem. 2015; 63: 4954-4965 (DOI:10.1021/acs.jafc.5b00951)

DOI 10.1021/acs.jafc.5b00951

Jacchia S, Nardini E, Savini C, Petrillo M, Angers-Loustau A, Shim J-H, Trijatmiko K, Kreysa J, Mazzara M. Development, optimization, and single laboratory validation of an event-specific real-time PCR method for the detection and quantification of Golden Rice 2 using a novel taxon-specific assay. J. Agric. Food Chem. 2015; 63:

DOI 10.1021/jf505516y

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)	3' integration border region (IBR) between the insert of rice event Golden Rice 2 and the rice host genome
Primer Forward	5'-TGGCCGTATCCGCAATGTG-3'
Target element	insert
Primer Reverse	5'-GCTGCGCCTCTAACCAGGTAT-3'
Target element	3'-host genome
Amplicon length	121 bp
Probe	5'-FAM-TCGATATGATTCCTTCATCGAGCGAGC-TAMRA-3'

Taxon-target(s)	phospholipase D alpha 2 (PLD) gene
Primer Forward	5'-GGCAAGAACAACACCATTGACA-3'
Target element	PLD α2
Primer Reverse	5'-CGGCTTTCCATGCAAATGA-3'
Target element	PLD α2
Amplicon length	124 bp
Probe	5'-FAM-ATTCGCCGCGCCAAGAACTTCA-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	0.30 µmol/L	Primer Fw	0.30 µmol/L
Primer Rev	0.30 µmol/L	Primer Rev	0.30 µmol/L
Probe	0.15 µmol/L	Probe	0.15 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	5 µL	Template DNA	5 µL
Final Volume	25 µL	Final Volume	25 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