Rice quantitative PCR methods

Quantitative PCR method for detection of rice event LLRICE62

1. GENERAL INFORMATION

Target genetic element	3' integration border region (IBR) between the insert of rice event LLRICE62 and the rice host genome
PCR Assay	Simplex Real Time
Detection Chemistry	TaqMan®
Compendium Reference	QT-EVE-OS-002

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 rice event LLRICE62

Tested GM events

Event Name	LLRICE62
Unique Identifier	ACS-OS002-5
Crop Name	*Oryza sativa* L.

Collaborative Trial Description

The participants received 20 unknown samples representing five GM levels, namely 0.15%, 0.4%, 0.9%, 2.0% and 3.3% of rice event LLRICE62 DNA in non-GM rice DNA. In addition the laboratories received five calibration samples, amplification reagent controls, reaction reagents, primers and probes for the phospholipase D (*PLD)* reference gene and for the LLRICE62 specific system. Four replicates for each GM level were analysed in two runs with both the reference and the transgenic specific system. The ΔCt method was followed to calculate the GM content of the blind samples.

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 (%)	0.15%	0.40%	0.90%	2.0%	3.3%
Mean Value (%)	0.13%	0.37%	0.84%	1.9%	3.2%
RSD_r (%)	21%	12%	11%	9.8%	12%
RSD_R (%)	22%	14%	17%	12%	15%
Bias %	-11%	-7.4%	-7.1%	-3.2%	-2.4%

	GMO Target
Mean Slope	-3.3
Mean PCR Efficiency %	98
Mean R^2	0.99

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

3. REFERENCES

Mazzara M, Grazioli E, Savini C, Van Den Eede G. Event-specific Method for the Quantitation of Rice Line LLRICE62 Using Real-time PCR -Validation Report and Protocol - Sampling and DNA Extraction of Rice. EUR 22490 EN. 2006. JRC34091 (ISBN 92-79-03129-5)

4. PRIMERS AND PROBES SEQUENCES

GM-target(s)

Primer Forward	5'-AGCTGGCGTAATAGCGAAGAGG-3'
Target element	Insert
Primer Reverse	5'-TGCTAACGGGTGCATCGTCTA-3'
Target element	3'-host genome
Amplicon length	88 bp
Probe	5'-FAM-CGCACCGATTATTTATACTTTTAGTCCACCT-TAMRA-3'
Probe Name	TM019
Target element	DNA sequence in the 3' IBR

Taxon-target(s)

Primer Forward	5'-TGGTGAGCGTTTTGCAGTCT-3'
Target element	PLD
Primer Reverse	5'-CTGATCCACTAGCAGGAGGTCC-3'
Target element	PLD
Amplicon length	68 bp
Probe	5'-FAM-TGTTGTGCTGCCAATGTGGCCTG-TAMRA-3'
Probe Name	TM013
Target element	phospholipase D (*PLD*) gene

5. PCR REACTIONS SETUP

GM-target(s)		**Taxon-target(s)**	
Reagent	**Final Concentration**	**Reagent**	**Final Concentration**
TaqMan® Universal PCR Master Mix	1x	TaqMan® Universal PCR Master Mix	1x
Primer Fw	0,40 µmol/L	Primer Fw	0,20 µmol/L
Primer Rev	0,40 µmol/L	Primer Rev	0,20 µmol/L
Probe	0,20 µmol/L	Probe	0,20 µmol/L
Nuclease-free water	#	Nuclease-free water	#
Template DNA	maximum 200	Template DNA	maximum 200
Final Volume	25 µL	Final Volume	25 µL

6. AMPLIFICATION CONDITIONS

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

Stage	**Temperature**	**Time**	**No Cycles**
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