

PCR protocol

Method: QL-CON-35S166-F/hpt166-R

Qualitative real-time PCR (TaqMan) method for detection of the junction between the CaMV 35S promoter and the *hpt* gene.

Target: Target is the junction region between the Cauliflower mosaic virus (CaMV) 35S promoter (P-35S) and the *hygromycin phosphotransferase II (hpt)* in GM Kefeng6-like rices.

Table 1 - oligonucleotides

Name	DNA sequence of the oligonucleotides	Final concentration in PCR
target	P35S-hpt (90 bp)	
35S166-F	5' - gctgaaatcaccagtctctctctaca - 3'	900 nM
hpt166-R	5' - tgagttcaggcttttcatatctcat - 3'	600 nM
35S166-Tm	5' - YAK - tatctctctcgagctttcgcagatccgg - BBQ -3' ^a	100 nM

^aequivalent reporter dyes and/or quencher can be used.

Amplicon length: 90 bp

Amplicon sequence: 5'-

gctgaaatcaccagtctctctctacaaatctatctctctcgagctttcgcagatccggggggcaatgagatatgaaaa
agcctgaactca-3'

Table 2 – addition of reagents

Total reaction volume	25 µl
sample DNA (up to 200 ng) or control DNA	5 µl
Taqman universal Mastermix; PCR buffer solution ¹ (including MgCl ₂ , dNTPs, and DNA polymerase)	12,5 µl
35S166-F and hpt166-R	see Table 1
35S166-Tm	see Table 1
water	add to 25 µl
¹ brand of commercial PCR master mix and polymerase (optional)	

Table 3 – temperature-time program

step	parameter		temperature [°C]	time [s]	fluorescence measurement	cycles
1	initial denaturation		95	600	No	1
2	amplification	denaturation	95	15	No	45
		annealing/elongation	60	60	Yes	

3	final elongation	-	-	-	-
---	------------------	---	---	---	---

Method performance:

Table 4 - specificity

detection	experimentally verified (species/GMO)	theoretically verified (GenBank Acc.No.)
positive	Gm-Reis Kefeng6	
negative	Gm-Mais Bt176; Bt11; Mon810; GA21; Nk603; Mon863; TC1507; MIR604; 59122; Starlink-Mais (CBH 351) Gm-Soja RR-Soja 40-3-2 Gm-Raps MS8/RF3 Falcon GS40/90 Gm-Reis BT63; KMD1 Gm-Papaya <i>Sunup 55-1</i>	

Limit of Detection (LOD): 5-10 Copies; verified using Plasmid DNA with the cloned amplified sequence

Additional performance parameter for quantitative PCR methods:

LOQ: not available; due to the lack of certified positive reference material

Additional performance parameter for fully validated PCR methods:

False positive rate: not available

False negative rate: not available

RSD_R: not available

References:

Reiting R., Grohmann L., Mäde D. (2010) A testing cascade for the detection of genetically modified rice by real-time PCR in food and its application for detection of an unauthorized rice line similar to KeFeng6. J. Verbr. Lebensm. 5: 185-188.