

**Supplementary Table 1.** List of oligonucleotide primers and probes used in this study

Target	Name	Nucleotide sequences (5'→3')*
SSIIb (Maize endogenous)	SSIIb3-5'	CCAATCCTTGACATCTGCTCC
	SSIIb3-3'	GATCAGCTTGGGTCCGGA
	SSIIb-Taq	FAM-AGCAAAGTCAGAGCGCTGCAATGCA-TAMRA
P35S	P35S 1-5'	ATTGATGTGATATCTCCACTGACGT
	P35S 1-3'	CCTCTCCAAATGAAATGAACCTCCT
	P35S-Taq	FAM-CCCCTATCCTCGCAAGACCCTTCCT-TAMRA
GA21	GA21-3-5'	GAAGCCTCGGCAACGTCA
	GA21-3-3'	ATCCGGTTGAAAGCGACTT
	GA21-Taq	FAM-AAGGATCCGGTGCATGGCCG-TAMRA
MIR162	MIR162-f1	GCGCGGTGTCATCTATGTTACTAG
	MIR162-r1	TGCCTTATCTGTTGCCTTCAGA
	MIR162-p1	FAM-TCTAGACAATTCACTACATAAAAACGTCCGCCA-TAMRA
MIR604	MIR604 primer F	GCGCACGCAATTCAACAG
	MIR604 primer R	GGTCATAACGTGACTCCCTTAATTCT
	MIR604 probe	FAM-AGGCGGAACGACAATCTGATCATG-TAMRA
Le1 (Soybean endogenous)	Le1n 02-5'	GCCCTCTACTCCACCCCCCA
	Le1n 02-3'	GCCCATCTGCAAGCCTTTT
	Le1-Taq	FAM-AGCTTCGCCGCTTCAACTTCAC-TAMRA
RRS	RRS 01-5'	CCTTAGGATTCAGCATCAGTGG
	RRS 01-3'	GAATTGTCGCCGGGAATG
	RRS-Taq	FAM-CGCAACCGCCCCGAAATCC-TAMRA
LLS	KVM175	GCAAAAAAGCGGTTAGCTCCT
	SMO001	ATTCAGGCTGCGCAACTGTT
	TM031	FAM-CGGTCCTCCGATGCCCTCC-TAMRA
RRS2	MON89788-F	TCCCGCTCTAGCGCTTCAAT
	MON89788-R	TCGAGCAGGACCTGCAGAA
	MON89788-P	FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA

\*FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine

**Supplementary Table 2.** List of PCR conditions used in this study

PCR instruments	Target	PCR condition	
7900HT or 7500 Fast (Standard ramp speed)	MIR604	Pre-denaturation	50°C, 2 min
			95°C, 10 min
	Others	Denaturation	95°C, 15 sec
		Annealing/Extension	60°C, 1 min
Cycle		45 cycles	
7500 Fast (Fast ramp speed)	MIR604	Pre-denaturation	50°C, 2 min
			95°C, 10 min
	Others	Denaturation	95°C, 30 sec
		Annealing/Extension	59°C, 1 min
Cycle		45 cycles	
	MIR604	Pre-denaturation	95°C, 20 sec
		Denaturation	95°C, 6 sec
	Others	Annealing/Extension	60°C, 30 sec
		Cycle	45 cycles
	MIR604	Pre-denaturation	95°C, 20 sec
		Denaturation	95°C, 6 sec
	Others	Annealing/Extension	59°C, 30 sec
		Cycle	45 cycles

**Supplementary Table 3.**  $C_f$  values for the real-time PCR instruments described in the official detection methods for the authorized GM maize and soybean in Japan<sup>17)</sup>

Target	PCR instruments					
	7900HT	7500	LightCycler 480	LightCycler 96	QuantStudio 5	QuantStudio 12K Flex
P35S	0.38	0.46	0.41	0.41	0.43	0.40
GA21	1.99	2.13	2.19	2.17	1.55	1.61
MIR162	0.70	0.64	0.56	0.59	0.71	0.66
MIR604	0.44	0.44	0.44	0.43	0.44	0.44
RRS	1.04	1.02	0.96	0.90	0.97	1.00
LLS	0.98	0.98	1.07	1.11	1.08	1.10
RRS2	1.32	1.33	1.30	1.29	1.51	1.51