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ARTICLE

An Event-Specific DNA Microarray To Identify Genetically Modified Organisms in Processed Foods

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We developed an event-specific DNA microarray system to identify 19 genetically modified organisms (GMOs), including two GM soybeans (GTS-40-3-2 and A2704-12), thirteen GM maizes (Bt176, Bt11, MON810, MON863, NK603, GA21, T25, TC1507, Bt10, DAS59122-7, TC6275, MIR604, and LY038), three GM canolas (GT73, MS8×RF3, and T45), and one GM cotton (LLcotton25). The microarray included 27 oligonucleotide probes optimized to identify endogenous reference targets, event-specific targets, screening targets (35S promoter and *nos* terminator), and an internal target (18S rRNA gene). Thirty-seven maize-containing food products purchased from South Korean and US markets were tested for the presence of GM maize using this microarray system. Thirteen GM maize events were simultaneously detected using multiplex PCR coupled with microarray on a single chip, at a limit of detection of approximately 0.5%. Using the system described here, we detected GM maize in 11 of the 37 food samples tested. These results suggest that an event-specific DNA microarray system can reliably detect GMOs in processed foods.

KEYWORDS: Genetically modified food; event-specific; microarray; multiplex PCR

INTRODUCTION

Since the first genetically modified organism (GMO) was grown commercially in 1996, GM crop cultivation has expanded to approximately 125 million hectares in 25 countries in 2008. GM soybean was reported to occupy 65.8 million hectares, 53% of the global biotech area, followed by maize (37.3 million hectares, 30%), cotton (15.5 million hectares, 12%) and canola (5.9 million hectares, 5%) (*1*). Fifty-five countries have granted regulatory approval to import GM crops for food and feed, and to release GMO into the environment since 1996. As of January 2010, the Korea Food and Drug Administration (KFDA) had authorized use of 62 varieties of soybean, corn, canola, cotton, potato, alfalfa, and sugar beet.

As the number of GMOs increases, so does commitment to develop sensitive and specific systems that can simultaneously identify multiple target genes (*2*). At present, the polymerase chain reaction (PCR) is the technology used most widely to detect GMO. Multiplex PCR has been specially adapted to detect several GMOs simultaneously in a single tube (*3*−*11*). A PCR-based method that can distinguish one particular variety from others may be described as "gene-specific", "construct-specific", or "event-specific". The most specific of these, "event-specific PCR", uses primer pairs that identify the integration junction sequences placed between the host plant genome and the integrated foreign gene.

Recent developments in GMO detection include the combination of multiplex PCR with a DNA microarray, as reported by Rudi et al. (*12*). Bordoni et al. (*13*) detected and quantified GM maize (Bt-176 transgenic maize) using a ligation detection reaction combined with universal array technology. Germini et al. (*14*) developed a peptide nucleic acid array to detect GMOs in foods. Xu et al. (*15*) described a rapid and reliable system to detect and identify GM events using multiplex PCR coupled with an oligonucleotide microarray. Leimanis et al. (*16*) reported a microarray-based detection system for nine GMOs. Xu et al. (*17*) coupled multiplex PCR with an oligonucleotide microarray that included 20 probes for GM soybean and maize. Others developed an event-specific microarray method to detect seven GM soybean and maize varieties (*18*). More recently, Morisset et al. (*19*) reported a target amplification strategy that allows quantitative on-chip GMO detection. Schmidt et al. (*20*) developed a detection method for GM canola using multiplex PCR coupled with oligonucleotide microarray hybridization.

Despite the expense of equipment and need for specialized training, researchers often apply microarray technology to GMO detection because it is flexible and can detect multiple GMO varieties. However, most of the systems reported so far have been used to detect genetic modifications in seeds and grains. From rising concern for accidental or intentional introduction of GMOs into food ingredients stems the need for a method to detect GMOs in processed foods. Conventional microarray−PCR combinations do not reliably achieve this, because food processing may severely degrade target biomarkers, i.e., the genomic DNA of GMO. Moreover, PCR inhibitors naturally present in foods or formed in processing may hinder target gene amplification and detection by conventional protocols.

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Table 1. Primer Pairs Used in the Study and Amplification Lengths for Each Target Gene

primer name	sequence (5′ → 3′)	target	amplicon size (bp)
18SR-AF	GAAACGGCTACCACATCCAA	18S rDNA	101
18SR-AR	CACTAACGCGCCCGGTATTG		
P-35S-AF	AAGATGCCTCTGCCGACAGT	35S promoter	142
P-35S-AR	GATTGTGCGTCATCCCTTAC		
T-NOS-AF	GAATCCTGTTGCCGGTCTTG	*nos* terminator	194
T-NOS-AR	CCGCGCGCGATAATTTATCCTAGT		
Lec-AF	TCTCCGATGTGGTCGATTTG	lectin	100
Lec-AR	ACGTCATGCGATTCCCCAGG		
SSIIb-AF	GTACCGGAACTACAAGGAGA	*zSS*IIb	100
SSIIb-AR	GAGCACGTCCTCATACAGCA		
FatA-AF	GTTAATCGCCGTACCATCGC	*FatA*	100
FatA-AR	GGCTGATGCTTCCCTGATCC		
Acp1-AF	CGTGGATAAGGTATGTGAAG	*Acp*1	99
Acp1-AR	GAATCAGCTCCAAGATCAAG		
SPS-AF	CGGACACCGTCGGTGAATTG	*SPS*	98
SPS-AR	GTTCTCGTCGGACCAGCTGA		
RRS-AF	CGATTTCGGCAATGCCGCCA	GTS-40-3-2	139
RRS-AR	CTATTCACCAGTAACAGCAG		
A270412-AF	GGCGTTCGTAGTGACTGAGG	A2704-12	159
A270412-AR	AACCCTGGCGTTACCCAACT		
Bt176-AF	CATGACGTGGGTTTCTGGCA	Bt176	135
Bt176-AR	AACGGTCGGCCAAGGCTTCA		
Bt11-AF	GGCCATTTATCATCGACTTCC	Bt11	179
Bt11-AR	TGATCCGGCAAACAAACCAC		
M810-AF	AACGTGCCCGGTACTGGTTC	MON810	167
M810-AR	GACTGCTCGCAAGCAAATTC		
M863-AF	CATTTGTAGGTGCCACCTTC	MON863	176
M863-AR	CGGAGAGCACTTGTTGGGTT		
NK603-AF	TCGGCCAGCAAGCCTTGTAG	NK603	120
NK603-AR	GGACTATCCCGACTCTCTTC		
GA21-AF	TCCGCCGTTGCTGACTGCTT	GA21	148
GA21-AR	TGTAACCGTAGTCCCGCAGG		
T25-AF	GTCGATTCCACCCTCATACT	T25	141
T25-AR	CACTTTTCGGGGAAATGTGC		
TC1507-AF	TCAGACGATGGTCTCCGCCT	TC1507	125
TC1507-AR	CCCCTAAGACCGAGTACATA		
Bt10-AF	CAACCTCAGCAACCAACCAA	Bt10	167
Bt10-AR	TCGCCACTGACTACTTTACC		
DAS-AF	CGCACCTGTGATTGGCTCAT	DAS-59122-7	116
DAS-AR	GATTGTCGTTTCCCGCCTTC		
MIR604-AF	CGCTCTGCGCACGCAATTCA	MIR604	132
MIR604-AR	GGTTCTGTCAGTTCCAAACG		
TC6275-AF	AGATCTGCGCGCGATCGATA	TC6275	192
TC6275-AR	TCGGCGCGGTGCATTGCATA		
LY038-AF	TGGGTTCAGTCTGCGAATGT	LY038	141
LY038-AR	TGTCCAAGGATGGCACTCGG		
GT73-AF	CTGATCCATGTAGATTTCCC	GT73	118
GT73-AR	CTTCAGCAAGATTCTCTGTC		
MSRF-AF	CTTTGAGCCACTCGAAGGAC	MS8×Rf3	174
MSRF-AR	TCTTATCGACCATGTACTCG		
T45-AF	AGGGTTTCGCTCATGTGTTG	T45	174
T45-AR	CAACACGTGACTGTATTCCA		
LL25-AF	ACATCATCCGTTTCTTGGAC	LLcotton25	169
LL25-AR	GCAACTGTGCTGTTAAGCTC		

In this study, we developed a microarray-based method for GMO detection in processed foods. Our method included amplification of fragmented DNA, event-specific detection, and a multiplex assay covering a broad range of GMOs, incorporated into a practical food analysis protocol. The event-specific microarray detection method we developed used a total of 27 probes to detect 19 events in 4 GM crops. In addition, we tested 37 food samples containing maize to evaluate the microarray coupled with multiplex PCR.

MATERIALS AND METHODS

Samples. GM soybean (GTS-40-3-2 and A2704-12), GM maize (Bt176, Bt11, MON810, MON863, NK603, GA21, T25, TC1507, Bt10, DAS-59122-7, MIR604, TC6275, and LY038), GM canola (GT73, MS8×RF3, and T45), GM cotton (LLcotton25), and non-GMOs were provided by the Korea Food and Drug Administration (KFDA) and used as positive and negative controls for the microarray analysis. Thirty-seven food samples containing maize were purchased from Korean (27 samples) and US domestic markets (10 samples): nacho chips (7 brands), corn chip (1 brand), corn soup (6 brands), canned corn soup (1 brand), canned sweet corn (4 brands), pop corn (3 brands), corn flour (5 brands), corn bread (3 brands), corn bread mix (4 brands), corn starch (2 brands), and sausage containing corn (1 brand).

DNA Extraction. The GM crops were mixed with liquid nitrogen and ground using mortars and pestles. Water-containing food samples were lyophilized for two days to improve the purity and yield of DNA extracted

Table 2. Probe Sequences Used for the Microarray Hybridization

probe	sequence (5′ → 3′)
18S rRNA	CACTACCTCCCCGTGTCAGGATTGGGTAAT
P-35S	GAACGTCTTCTTTTTCCACGATGCTCCTCG
T-*nos*	CGGGACTCTAATCATAAAAACCCATCTCAT
lectin	TGGCAGCAGAGAACCCTATCCTCACCCACT
zSSIIb	CAGCTGAGGTCCTCGGCCATGCCGCGCGCC
FatA	GAGATAACTGCCCGTAGAGGATCTAAAGCC
Acp1	GTTGATTCACCGGTGATTGGTTTGTCATTG
SPS	TTCCTCTGGAACTTCTTCTTCATGGGAGTG
GTS 40-3-2	CATGATGCGCTTGAAATCGTAGACCCCGAC
A2704-12	CGACATAAGAAGAATAGAATGCTTGGCTGA
Bt176	ATGGCGTGCATCAATGGAGGAGAGAACATC
Bt11	GGGTCTGACGCTCAGTGGAACGAAAACTCA
MON810	AAAGAAGGCTACCGAAAGTCCTCGTTCAGG
MON863	TGACCCTACTTGTTCGGATGGGTGTTCACC
NK603	TGACCTCGAGTAAGCTTGTTAACGCGGCCG
GA21	GCAACAATGGTCTGCGGCAATGTACTCGGC
T25	CATGAGACAATAACCCTGATAAATGCTTCA
TC1507	AAGGTCCGATAGAAAGGTCGAGTCCGAGCC
Bt10	TCGTTGCTCTGAAGAACATGACACACAGGG
DAS-59122-7	TGCTTATCCCTTCACTCTTTCTTCCGTCCC
MIR604	TCATAACGTGACTCCCTTAATTCTCCGCTC
TC6275	GGATGCAAACGTACCGTAATAAATTGACGC
LY038	ACCCATAAACTCCGCTCGGATCCTGAGATT
GT73	GGCAAGGAAAGGAAGGAGGATGATCTTCAT
MS8×RF3	CGAGTACTGAAGAGAAAATTGCAAAGGTCG
T45	CATACTAAGGGTTTCTTATATGCTCAACAC
LLcotton25	GACGGCCGAGTACTGTTAAGCTCAGTTGAA

from the samples before being ground in liquid nitrogen. The DNeasy Plant Maxi kit (Qiagen, Hilden, Germany) and Wizard Magnetic DNA Purification System for Food (Promega, Madison, WI) were used to extract DNA according to the manufacturer's instructions. Ethanol precipitation with 3 M sodium acetate (pH 5.2) was used to concentrate the DNA if necessary. The concentration of genomic DNA was estimated using a UV spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan) and adjusted to 50 ng/μL.

Primers and Probes. The nucleotide sequences of the primers and probes are shown in **Tables 1** and **2**, respectively. Schematic diagrams in **Figure 1** show the location of each primer pair on the GM events and the flanking regions of genomic DNA. Twenty-seven specific primer pairs were designed for microarray analysis of 19 different GMOs. The target genes used in this study were as follows: endogenous reference genes; specific integration junction sequences between the host plant genome and the integrated gene; 35S promoter (P-35S) from cauliflower mosaic virus; nopaline synthase gene terminator (T-nos) from *Agrobacterium tumefaciens*; and 18S rDNA partial sequence. Lectin, maize starch synthase IIb (zSSIIb), fatty acyl-ACP thioesterase (FatA), acyl carrier protein (Acp1), and sucrose phosphate synthase (SPS) genes were chosen as endogenous reference genes of soybean, maize, canola, cotton, and rice, respectively. The forward primers, labeled at the 5′ end with fluorescent Cy3, were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). The amplicon size was kept to less than 200 bp for GMO analysis in processed foods. The 5′ amine-modified 30-mer oligonucleotides were synthesized as probes for microarray, complementary to the labeled PCR products by the Bionics Company (Seoul, Korea).

Single-Target PCR. PCR was performed in a thermal cycler (PC808, ASTEC, Kyoto, Japan). Each 25 μL reaction contained 2.5 μL of 10× buffer (Applied Biosystems, Foster City, CA), 200 μM each of dNTP (Applied Biosystems), 1.5 mM $MgCl_2$, 0.8 unit of Ampli Gold *Taq* DNA polymerase (Applied Biosystems), and 100 ng of template DNA. The concentration of each primer was 10 μM in a single-target PCR. The conditions for a single-target PCR were an initial denaturation at 94 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, and extension at 72 °C for 30 s, with a final elongation at 72 °C for 8 min.

DNA Sequencing. After the specificity of primer pairs was assessed using single-target PCR, the amplified PCR products were purified by QIAquick PCR purification kit (Qiagen), cloned into pGEM-T easy

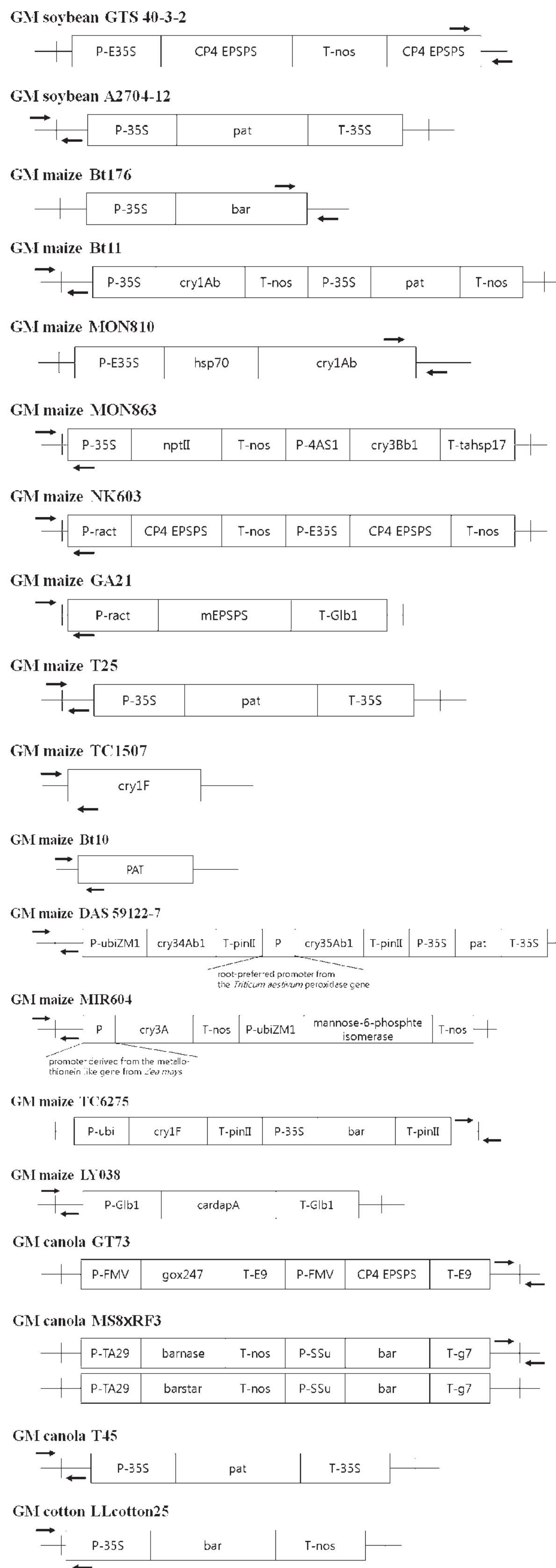


Figure 1. Schematic diagrams of the event-specific primers designed to detect nineteen different GMO events. The location of the primers used for the amplification is indicated by arrows. All the primer pairs were designed based on the sequences of the transferred DNA flanking region and the inserted region of the GMO genome, respectively in each event. The vertical lines represent the right or left borders.

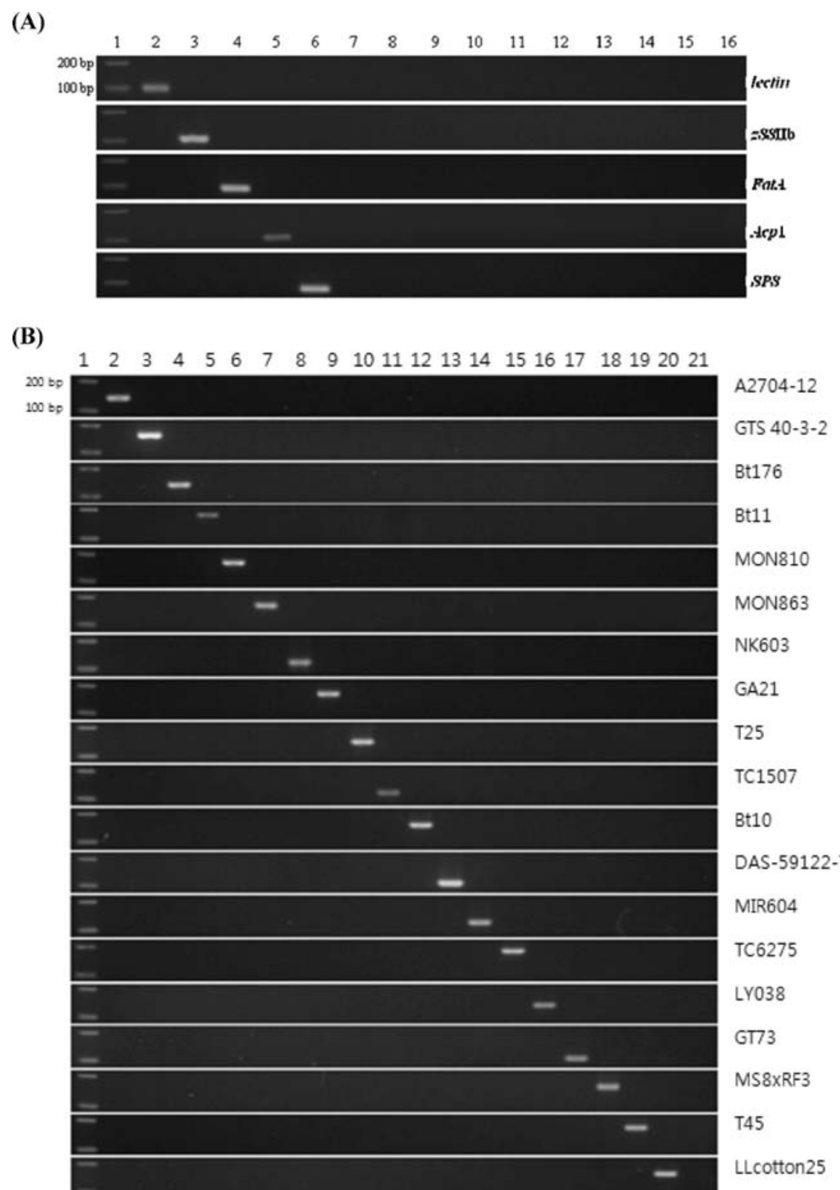


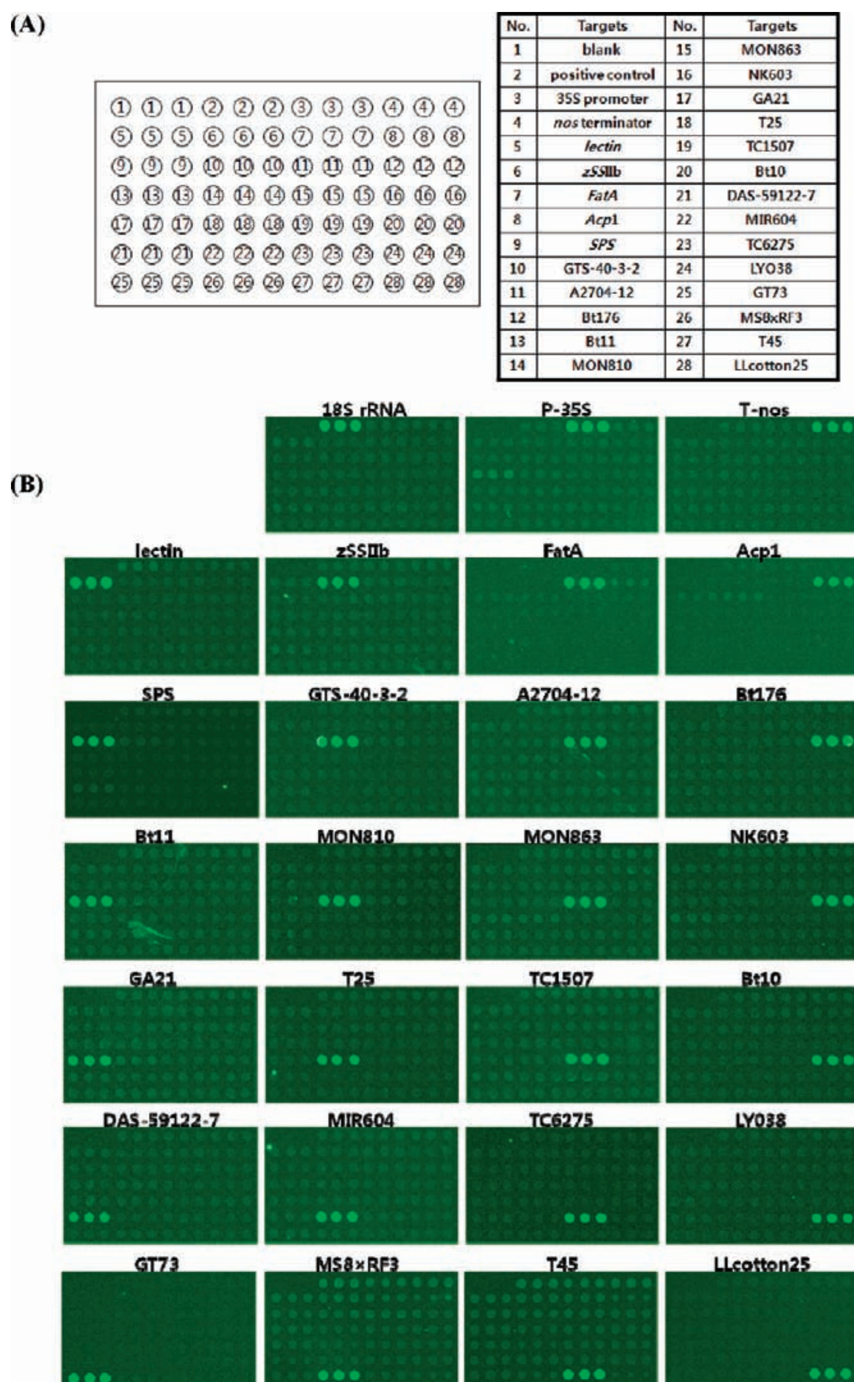
Figure 2. Specificity of the primer pairs for endogenous genes (**A**) and event-specific genes (**B**) in GMO reference materials used in this study. (**A**) Lanes 1−16: marker (100 bp DNA Ladder), soybean (lectin), maize (zSSIIb), canola (FatA), cotton (Acp1), rice (SPS), potato, barley, buckwheat, wheat, pepper, red bean, radish, Chinese cabbage, perilla leaf, and nontemplate. zSSIIb: maize starch synthase IIb. FatA: fatty acyl-ACP thioesterase. Acp1: acyl carrier protein. SPS: sucrose phosphate synthase. (**B**) Lane 1: marker (100 bp DNA ladder); lanes 2−21: GTS-40-3-2, A2704-12, Bt176, Bt11, MON810, MON863, NK603, GA21, T25, TC1507, Bt10, DAS-59122-7, MIR604, TC6275, LY038, GT73, MS8×RF3, T45, LLcotton25, and no template.

vector (Promega). DNA sequencing was performed using an ABI PRISM 3700 DNA analyzer (Perkin-Elmer, Boston, MA).

Microarray Preparation. All probes were diluted to a final concentration of 50 μM in hybridization solution (GenoCheck Co., Ansan, Korea) and then spotted onto CMT-GAPS II silane slide glass (Corning, Acton, MA) using a PixSys 5500 arrayer (Cartesian Technologies, Irvine, CA) with Stealth micro spotting pins approximately 100 μm in diameter. The microarray presented on the glass included 27 different probes (30-mer oligonucleotides). The printed slides were processed according to the CMT-GAPS II slide protocol. After processing, a random sampling of arrays was stained with Syto61 (Molecular Probes, Eugene, OR) to evaluate the quality of printing (*21*), and scanned with the GenePix 4100A scanner (Axon Instruments, Redwood City, CA).

Hybridization of PCR Products and Scanning. A CoverWell perfusion chamber (PC8R-0.5: Grace-Bio Laboratories, Bend, OR) was placed on the top of a glass slide to form a fixed volume (50 μL). Two microliters of PCR products for single PCR or 10 μL of PCR products for multiplex PCR were mixed with 46 or 38 uL of hybridization solution (GenoCheck), respectively. The mixtures (48 μL) were transferred to the placed CoverWell perfusion chambers. The arrays were then hybridized at 42 °C for 1 h in a humidified hybridization chamber (Array Chamber II, GenomicTree, Daejeon, Korea), and subsequently washed four times with washing solutions sequentially as follows: 2× SSC, 0.1% SDS at 42 °C for 30 min; 0.1× SSC, 0.1% SDS at room temperature (RT) for 20 min; 0.1× SSC at RT for 10 min; and 0.01× SSC at RT for 1 min. After drying, the washed arrays were scanned with a GenePix 4100A scanner (Axon Instruments) and the scanned images were analyzed with the GenePix Pro 6.0 software (Axon Instruments). Scanning was done with a pixel resolution of 10 μm, a laser power of 100%, and a PMT Gain at 600 V. The microarray assay was repeated three times.

Multiplex PCR Coupled with Microarray and Limit of Detection (LOD) with GM Maize Mixtures. Four sets of the multiplex PCR for 13 events of GM maize were designed and optimized to simultaneously detect each target gene using four arrays on a single chip, which contained 27 elements spotted in triplicate. DNA mixtures, containing three GM maizes (M1: MON863, TC1507, and MIR604), four GM maizes (M2: Bt176, MON810, NK603, and GA21), four GM maizes (M3: Bt10, DAS-59122-7, TC6275, and LY038), and two GM maizes (M4: Bt11 and T25),



No.	Targets	No.	Targets
1	blank	15	MON863
2	positive control	16	NK603
3	35S promoter	17	GA21
4	*nos* terminator	18	T25
5	*lectin*	19	TC1507
6	*zSSIIb*	20	Bt10
7	*FatA*	21	DAS-59122-7
8	*Acp1*	22	MIR604
9	*SPS*	23	TC6275
10	GTS-40-3-2	24	LY038
11	A2704-12	25	GT73
12	Bt176	26	MS8xRF3
13	Bt11	27	T45
14	MON810	28	LLcotton25

Figure 3. A schematic representation of the GMO chip contents (**A**) and specificity of the probes on the microarray system developed in this study (**B**). All probes were spotted in triplicate. The 18S rRNA probe was designed as a positive hybrid internal control, and P-35S and T-nos probes were designed as GMO screening elements. Lectin, starch synthase IIb (zSSIIb), fatty acyl-ACP thioesterase (FatA), acyl carrier protein (Acp1), and sucrose phosphate synthase (SPS) were also selected as endogenous references for soybean, maize, canola, cotton, and rice, respectively. GM crops used to design specific probes are GM soybean (GTS-40-3-2 and A2704-12), GM maize (Bt176, Bt11, MON810, MON863, NK603, GA21, T25, TC1507, Bt10, DAS-59122-7, MIR604, TC6275, and LY038), and GM canola (GT73, MS8×RF3, and T45), GM cotton (LLcotton25).

were prepared in our laboratory. The microarray assay of mixture M1 included five pairs of primers (1 μM 18S-AF/AR, 5 μM SSIIb-AF/AR, 30 μM M863-AF/AR, 30 μM TC1507-AF/AR, and 10 μM MIR604-AF/AR) corresponding to sequences of the 18S rRNA gene, *zSS*IIb, MON863, TC1507, and MIR604, respectively. For the microarray assay of mixture M2, PCR products were amplified by multiplex PCR using five pairs of primers (1 μM 18S-AF/AR, 3 μM Bt176-AF/AR, 10 μM M810-AF/AR, 3 μM NK603-AF/AR, and 15 μM GA21-AF/AR). For the microarray assay of mixture M3, fragments of the 18S rRNA gene, Bt10, DAS-59122-7, TC6275, and LY038 were simultaneously amplified by multiplex PCR using five pairs of primers (1 μM 18S-AF/AR, 20 μM Bt10-AF/AR, 10 μM DAS-AF/AR, 20 μM TC6275-AF/AR, and 10 μM LY038-AF/AR). The microarray assay of mixture M4 included three pairs of primers (0.5 μM 18S-AF/AR, 20 μM Bt11-AF/AR, and 20 μM T25-AF/AR) corresponding to sequences of the 18S RNA gene, Bt11, and T25. Each primer pair concentration was adjusted independently until the spot signals showed similar intensities on a scanned image. Multiplex PCR mixture contained the same PCR ingredients as single-target PCR. Multiplex PCR was composed of one cycle of initial denaturation at 94 °C for 5 min, 40 cycles of denaturation at 94 °C for 1 min, annealing at 61 °C

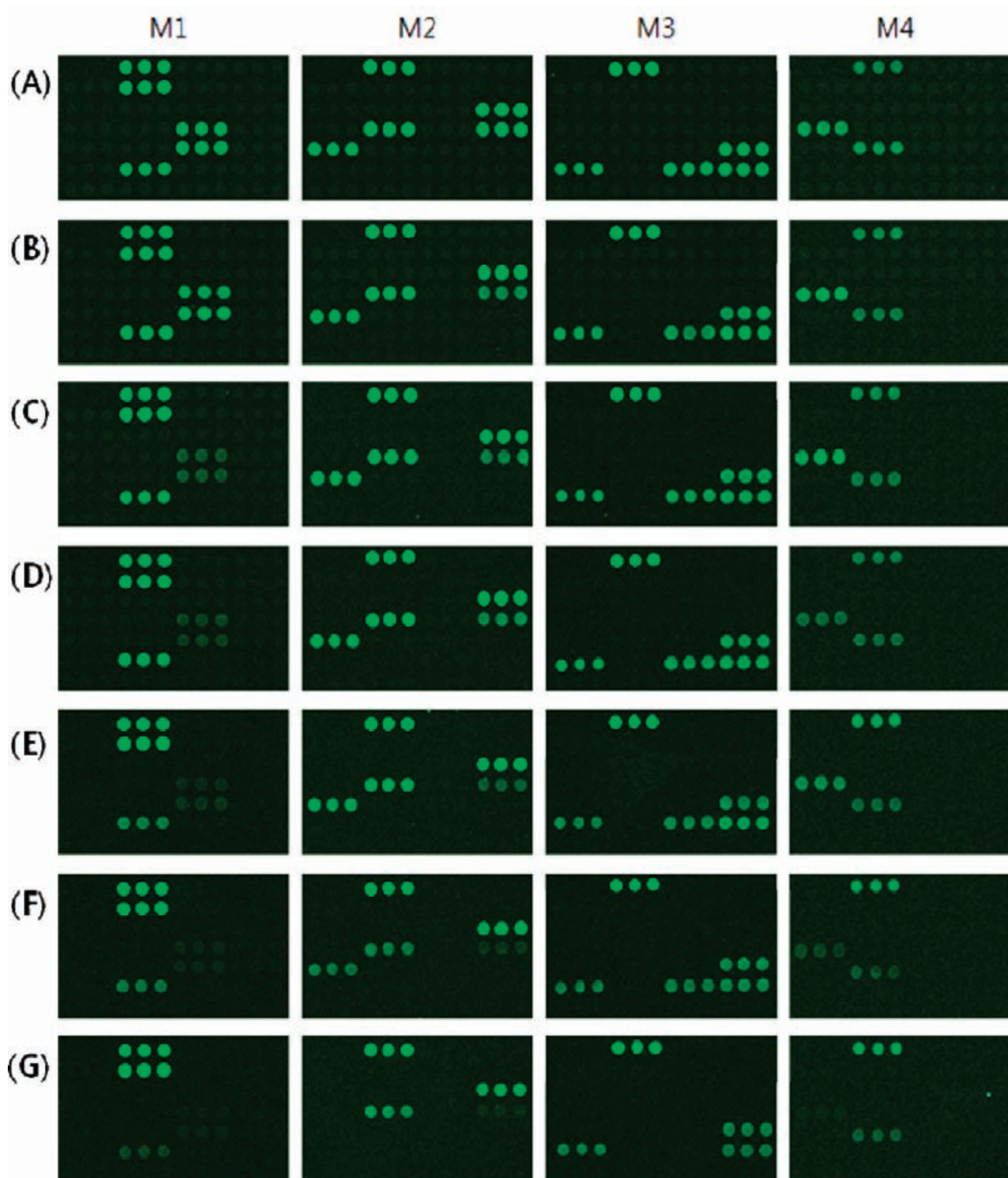


Figure 4. Limits of detection for four GM maize mixtures. (**A**) to (**G**) correspond to 100, 10, 5, 3, 1, 0.5, and 0.1% GM maize mixtures (M1, MON863, TC1507, MIR604; M2, Bt176, MON810, NK603, GA21; M3, Bt10, DAS-59122-7, TC6275, LY038; M4: Bt11, T25) used for microarray detection, respectively.

for 1 min, extension at 72 °C for 1 min, and a final elongation at 72 °C for 8 min. Microarray was performed to detect targets as described in previous sections.

The LOD of multiplex PCR coupled with microarray was determined for 13 GM maize events. Pure non-GM maize DNA was mixed with the four GM maize event mixtures (M1, M2, M3, and M4), described in a previous section; LOD mixtures were produced to contain 100, 10, 5, 3, 1, 0.5, and 0.1% (v/v) GM maize event mixture, M1 to M4. A total of 100 ng of DNA from each GM maize mixture was used as template in a reaction.

RESULTS

Specificity of Primers and Probes. The specificity of the designed primer pairs was individually assessed by single-target PCR. As shown in **Figure 2**, PCR products with the lengths expected for the target sequences in endogenous and event-specific gene were amplified from GMO reference material. Each PCR product of the single-target PCR was sequenced, and all the amplicons were found to have correct target gene sequence (data not shown).

The microarray format shown in **Figure 3A** included probes for GMO screening targets (P-35S and T-nos), endogenous reference targets (lectin, zSSIIb, FatA, Acp1, and SPS), event-specific targets, and an internal target (18S rDNA). The specificity of probes on the GMO chip was confirmed by hybridization of the corresponding PCR amplicons obtained from each target. Each probe showed a specific signal without cross-hybridization (**Figure 3B**).

Microarray and LOD with GM Maize Mixtures. As shown in **Figure 4**, the specific hybridization signals for maize endogenous genes, internal controls, and 13 events of GM maize were simultaneously detected on a single chip.

The sensitivity of the assay was also assessed by reference standard DNA mixtures ranging from 50 to 200 ng (50, 100, 150, and 200 ng) (data not shown). It was determined that 100 ng of DNA was the lowest concentration suitable for the simultaneous detection of 13 GM maize events without false negatives. In in-house validation of a microarray system for 13 events in GM maize, LOD was determined to be 0.5% (**Figure 4**).

Table 3. Microarray Detection of GM Maize in Processed Foods

		GM maize events													
sample no.	type of product	Bt176	Bt11	MON810	MON863	NK603	GA21	T25	TC1507	Bt10	DAS-59122-7	MIR604	TC6275	LY038	no. of GM maize
1	nacho chips A	−	−	−	−	−	−	−	−	−	−	−	−	−	0
2	nacho chips B	−	−	−	−	−	−	−	−	−	−	−	−	−	0
3	nacho chips C	−	−	+	−	+	−	−	−	−	+	−	−	−	3
4	nacho chips D	−	−	−	−	−	−	−	−	−	−	−	−	−	0
5	nacho chips E	−	−	−	−	−	−	−	−	−	−	−	−	−	0
6	nacho chips F	−	−	+	−	+	−	−	+	−	+	−	−	−	4
7	nacho chips G	−	−	−	−	−	−	−	−	−	−	−	−	−	0
8	corn chips	−	−	−	−	−	−	−	−	−	−	−	−	−	0
9	corn soup A	−	−	+	−	+	−	−	−	−	−	−	−	−	2
10	corn soup B	−	−	−	−	−	−	−	−	−	−	−	−	−	0
11	corn soup C	−	−	−	−	−	−	−	−	−	−	−	−	−	0
12	corn soup D	−	−	+	−	+	+	−	+	−	+	−	−	−	5
13	corn soup E	−	−	−	−	−	−	−	−	−	−	−	−	−	0
14	corn soup F	−	−	−	−	−	−	−	−	−	−	−	−	−	0
15	canned corn soup	−	−	−	−	−	−	−	−	−	−	−	−	−	0
16	canned sweet corn A	−	−	−	−	−	−	−	−	−	−	−	−	−	0
17	canned sweet corn B	−	−	−	−	−	−	−	−	−	−	−	−	−	0
18	canned sweet corn C	−	−	−	−	−	−	−	−	−	−	−	−	−	0
19	canned sweet corn D	−	−	−	−	−	−	−	−	−	−	−	−	−	0
20	popcorn A	−	−	−	−	−	−	−	−	−	−	−	−	−	0
21	popcorn B	−	−	−	−	−	−	−	−	−	−	−	−	−	0
22	popcorn C	−	−	−	−	−	−	−	−	−	−	−	−	−	0
23	corn flour A	−	−	−	−	+	−	−	−	−	−	−	−	−	1
24	corn flour B	−	−	+	−	−	−	−	−	−	−	−	−	−	1
25	corn flour C	−	−	−	−	−	−	−	−	−	−	−	−	−	0
26	corn flour D	−	+	+	+	+	+	+	+	−	+	+	−	−	9
27	corn flour E	−	−	−	−	−	−	−	−	−	−	−	−	−	0
28	corn bread A	−	−	−	−	−	−	−	−	−	−	−	−	−	0
29	corn bread B	−	−	−	−	−	−	−	−	−	−	−	−	−	0
30	corn bread C	−	−	−	−	−	−	−	−	−	−	−	−	−	0
31	corn bread mix A	−	−	−	−	−	−	−	−	−	−	−	−	−	0
32	corn bread mix B	−	−	−	−	−	−	−	−	−	−	−	−	−	0
33	corn bread mix C	−	−	+	−	+	−	−	+	−	+	−	−	−	4
34	corn bread mix D	−	+	+	−	+	+	+	+	−	+	−	−	−	7
35	corn starch A	−	−	+	−	−	−	−	−	−	−	−	−	−	1
36	corn starch B	−	−	+	−	+	−	−	+	−	−	−	−	−	3
37	sausage containing corn	−	−	−	−	−	−	−	−	−	−	−	−	−	0

Detection of GM Maize in Processed Foods Using Microarray. This microarray analysis of 37 maize-containing food samples available in the market identified GM maize in 11 different samples (**Table 3**). Sample 3 contained three GM events (MON810, NK603, and DAS-59122-7); samples 6 and 33, four events (MON810, NK603, TC1507, and DAS-59122-7); sample 9, two events (MON810 and NK603); sample 12, five events (MON810, NK603, GA21, TC1507, and DAS-59122-7); sample 23, one event (NK603); samples 24 and 35, one event (MON810); sample 26, nine events, (Bt11, MON810, MON863, NK603, GA21, T25, TC1507, DAS-59122-7, and MIR604Bt11, and T25); sample 34, seven events (Bt11, MON810, NK603, GA21, T25, TC1507, and DAS-59122-7); and sample 36, three events (MON810, NK603, and TC1507). Four of eleven positive samples (sample No. 6, 26, 33, and 34) were purchased from US food markets, and the other seven (sample No. 3, 9, 12, 23, 24, 35, and 36) were bought from Korean food markets. It was found that all the samples except sample No. 24 were made of US maize; sample No. 24 was produced using Korean maize.

DISCUSSION

Degradation of DNA by chemical, physical, and enzymatic factors in food processing will influence the accuracy of a PCR method for GMO analysis. The size of PCR amplicon may be particularly important in this respect. In this study, all the primer pairs were designed to generate amplicons smaller than 200 bp, and this proved to be suitable for GMO detection in processed foods. Depending on food processing, DNA extracted from food samples may not be detected on agarose gel, so the amount of DNA can be insufficient as templates of multiplex PCR. After DNA extraction, the DNA from several corn starch and corn chips for PCR should be concentrated to be higher than 50 ng/μL. The A_{260}/A_{280} ratio of the genomic DNA extracted from processed foods was within a range of 1.58 to 1.92; the extracted DNA was pure enough to perform PCR.

Multiplex PCR may provide the most straightforward, rapid, and cost-effective approach to simultaneous detection of GMOs. However agarose gel electrophoresis does not easily resolve PCR products of similar length or large numbers of GM events (*17*). For high-throughput GMO analysis, alternative methods are therefore required. Microarray hybridization meets these requirements and is also very flexible, in that new GMO varieties can be included in the screening procedure simply by adding new probe sequences to the array (*22*). The compatibility of primer pairs, and the specificity and sensitivity of hybridization between PCR products and probes, substantially influence the validity of microarray experiments. In this study, as the first optimization step of the event-specific multiplex PCR, each primer pair was designed to have similar T_m values and G + C contents (%). The specificity of each primer pair to individual target GM events was confirmed (**Figure 2**), and then the primers were used for the multiplex PCR. In addition, the probe sequences were designed to be

complementary to internal sequences of the PCR amplicons and to avoid self-complementarity and the formation of hairpin loops (**Table 2**). The probes used in this study showed no cross-hybridization with unrelated amplicons on the microarray chip, confirming their specificity. The efficiency of PCR and/or hybridization may also influence the LOD of the microarray. For example, the binding affinity between a target and probe DNA correlates directly or indirectly with LOD values. Moreover, a variety of factors should be considered to improve the sensitivity of the microarray system: consistent spot diameter and morphology; primers and template DNA concentrations of multiplex PCR; amplicon amounts and probe concentration for hybridization. The LOD value obtained in this study (0.5%) complies with labeling threshold levels in South Korea (3%), Japan (5%), and the EU (0.9%) (*23−26*).

The microarray system devised in this study contained 18S rRNA gene as an internal control to assess the efficiency of all the reactions and to eliminate false negatives (*15*, *17*). More than 95% of the presently available GMO crops are positive for either P-35S or T-nos, or both applications, showing that these provide reliable markers for GMO screening (*23*, *27*). Therefore, P-35 and T-nos were also used as screening elements for GMO detection in this study. The threshold levels of the positives and negatives for spot signals of the microarray were determined by the intensity of each spot, calculated by averaging the values of all pixels within the spot boundaries. Spot signal intensities were corrected by subtraction of the background values. The threshold level for a positive signal was determined to be at least 10-fold higher than the blank. Every experiment was repeated three times. To determine the level of false positives, each food sample containing GM maize was retested using single-target PCR. We found no false positive results (data not shown).

Event-specific PCR detection methods are preferred to eliminate the possibility of false positives. Reliability and reproducibility of the methods should be confirmed through repeated experiments from sample preparation to data analysis. Moreover, the possibility of carryover contamination caused by separation of PCR and hybridization steps should be carefully considered because it can result in false positive results (*27*). Climate controlled facilities and favorable environment for microarray construction may maintain consistency of the results (*20*). Consequently, event-specific PCR coupled with microarray may be an ideal analytical tool for GMO detection by yielding the fewest false positives.

As new GMOs, including "stacked-gene" varieties, are continually developed, the corresponding detection tools are required to monitor them. Although in this study we used microarray technology as a first-line screening assay only, the same methodology may in the future be used to both detect and quantify new GMO varieties. We found that a single microarray chip can analyze 19 different GMOs, which supports the further development of our system for microarray-based detection of various GM crops.

In conclusion, we developed an event-specific DNA microarray detection system for 19 different GMOs, and showed that the system can simultaneously detect 13 events in GM maize using a single chip. To the best of our knowledge, this is the first successful demonstration of a microarray system to simultaneously detect 13 GM maize events in a variety of maize-containing processed foods. We applied this new methodology to 37 maize-containing food samples and demonstrated its potential for broader application. We are currently extending the capacity of this event-specific DNA microarray system to cover more GM events, and expect to apply it soon to food samples containing soybean, maize, potato, rice, and wheat.

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