JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Practical Experiences with an Extended Screening Strategy for Genetically Modified Organisms (GMOs) in Real-Life Samples

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Supporting Information

ABSTRACT: Nowadays most animal feed products imported into Europe have a GMO (genetically modified organism) label. This means that they contain European Union (EU)-authorized GMOs. For enforcement of these labeling requirements, it is necessary, with the rising number of EU-authorized GMOs, to perform an increasing number of analyses. In addition to this, it is necessary to test products for the potential presence of EU-unauthorized GMOs. Analysis for EU-authorized and -unauthorized GMOs in animal feed has thus become laborious and expensive. Initial screening steps may reduce the number of GMO identification methods that need to be applied, but with the increasing diversity also screening with GMO elements has become more complex. For the present study, the application of an informative detailed 24-element screening and subsequent identification strategy was applied in 50 animal feed samples. Almost all feed samples were labeled as containing GMO-derived materials. The main goal of the study was therefore to investigate if a detailed screening strategy would reduce the number of subsequent identification analyses. An additional goal was to test the samples in this way for the potential presence of EU-unauthorized GMOs. Finally, to test the robustness of the approach, eight of the samples were tested in a concise interlaboratory study. No significant differences were found between the results of the two laboratories.

KEYWORDS: *GMO element, screening plate, multidetection, unauthorized GMO, TaqMan, PCR, Cry3Bb1, Cry1F*

INTRODUCTION

For routine genetically modified organism (GMO) analysis of food and feed products, the polymerase chain reaction (PCR) is, in Europe, the method of choice. GMO analysis comprises DNA isolation followed by screening for a limited number of GMO-derived elements that occur in a wide range of GMOs. In this way the sample can be assessed for the potential presence of GMOs in a cost-effective way. The selection of screening elements should be such that the number of false-negative samples is minimal. On the basis of the results of this initial screening step, construct- or event-specific methods are applied to identify the GMOs involved. A construct-specific method amplifies a sequence bridging two elements within a GMO, but this same combination of elements may also occur in other GMOs. An event-specific method amplifies a specific sequence spanning the border between the plant DNA and the DNA of the insert. This method will specifically identify a GMO as integration in the plant is still random and thus unique for each given GMO.

For all GMOs authorized in the European market validated event-specific methods developed by the producer of the GMO are available on the Internet.[1] The development of event-specific methods, however, requires prior sequence knowledge of the GMO to be identified. This information is usually not available for EU-unauthorized GMOs. In those cases, a combination of GMO-derived elements and identified EU-authorized GMOs may indicate, by unexplained GMO elements, the potential presence of unauthorized GMOs in a given sample. As a result of the increased diversity of genetic elements used in GMOs, it has become necessary to use a broad range of screening elements to enable the detection of all EU-authorized and, based on the selected screening elements, at least some of the EU-unauthorized GMOs that may enter our food and feed supply chains.

Several laboratories have developed screening strategies using either SYBRGreen[2] or TaqMan element-specific methods.[3] Waiblinger et al.[3] present a validated system for GMO element screening, detecting at least 81 both authorized and unauthorized GMOs. The system of Waiblinger et al. applies three element-specific (*P-35S*, *T-nos*, and *BAR*) and two construct-specific PCR methods (*ctp2-CP4-EPSPS* and *P35S-PAT*) with TaqMan probes. The advantage of using TaqMan probes is that this makes the PCR method highly sequence-specific. Thus, the PCR signals are easier to interpret than with SYBRGreen detection. However, if the sequence of the bases in the DNA between the forward and reverse primers is not identical for a given element in different GMOs, the element remains

Received: April 25, 2013
Revised: August 13, 2013
Accepted: August 21, 2013
Published: August 21, 2013

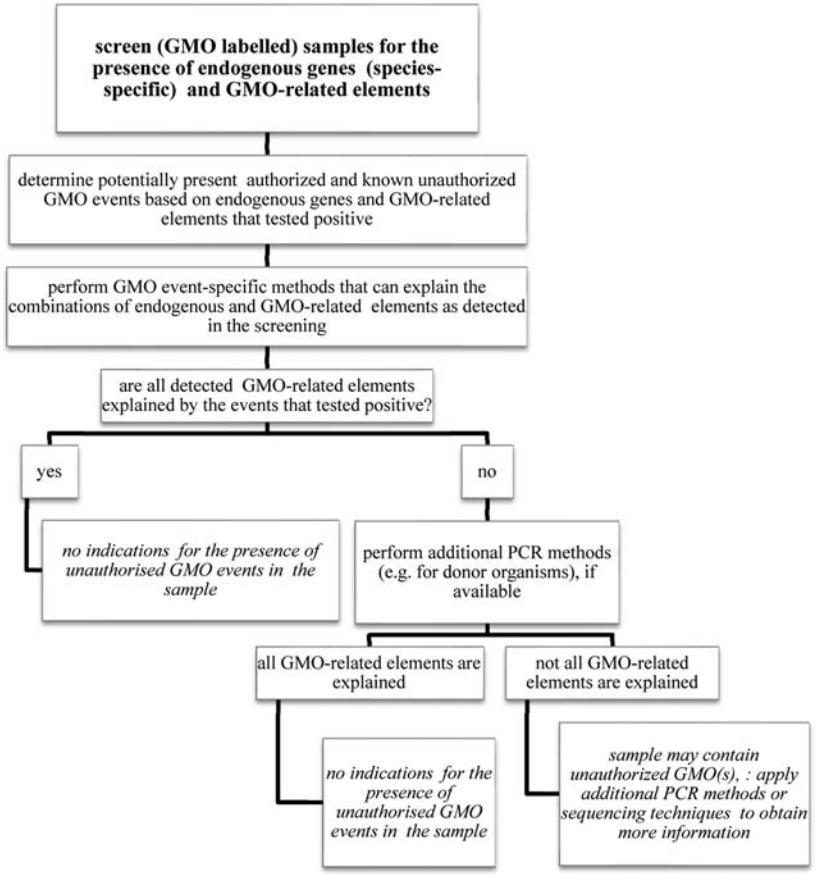

Figure 1. Schematic overview of the workflow for extended element screening of GMO-labeled animal feed samples.

undetected by the TaqMan probe in some GMOs. In that case it is necessary to develop separate TaqMan PCR methods for the different sequences of the element or, indeed, use SYBRGreen detection, as the latter allows interprimer sequence differences. There are, however, already a number of known authorized and unauthorized GMOs that will not be detected by the Waiblinger protocol, for example, MON89788 and DP305423 soy, and 281-24x3006-210-23 cotton. For GMO-labeled animal feed samples, which may contain several crops and GMOs, an extended element screening as proposed in this paper may lead to a reduction of event-specific tests that are needed for confirmation, compared to the more concise Waiblinger protocol.

Furthermore, in the case of positive screening elements, it will need to be established that the GMO element cannot be explained by the presence in the sample of similar elements derived from the unmodified plant or by the presence of, for example, external viral and/or bacterial agents. In all cases, additional sequence information will be needed to confirm the presence of any unauthorized GMO on the basis of this initial screening approach. A schematic overview of the screening strategy is presented in Figure 1.

In this study the extended element screening was applied on 50 animal feed samples taken from the Dutch official GMO monitoring program in feed materials. It was investigated whether such a detailed screening strategy may be of value and could reduce the number of subsequent identification analyses. Furthermore, the robustness of this system was tested with eight samples that were analyzed in two different laboratories on two different PCR platforms. The advantages and disadvantages of the extended GMO element screening approach for animal feed samples will be discussed.

MATERIALS AND METHODS

Samples. All samples were taken from the Dutch monitoring program for the presence of GMOs in food and feed.

DNA Extraction. Institute of Reference Materials and Measurements (IRMM) (Geel, Belgium) and American Oil Chemists' Society (AOCS) (Urbana, IL, USA) certified reference materials were used for DNA isolation without further preparation. Real-life samples of animal feed were milled through a 1 mm sieve on a Retsch ZM200 mill. DNA was isolated from 100 ±10 mg dry material of each sample using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturers' protocol. For maize, canola, and mixed samples the lysis step was carried out with cetyl trimethylammonium bromide (CTAB) extraction buffer (20 g/L CTAB, 1.4 M NaCl, 0.1 M Tris, 20 mM Na_2EDTA, pH 8.0) instead of the manufacturers' AP1 buffer (composition is not given in the Qiagen kit information). During incubation, 20 μL of 20 mg/mL Proteinase K was added to the isolation. The DNA concentrations were measured on a Thermo Scientific NanoDrop spectrophotometer. A different method for DNA isolation was used for the robustness tests at The Netherlands Food and Consumer Product Safety Authority (NVWA). DNA was isolated using the Promega Wizard DNA Clean-Up System according to the manufacturer's protocol. Both DNA extraction methods had been in-house validated and were accredited. DNAs extracted from reference materials were diluted with water (ultrapure distilled water, DNase and RNase free, Life Technologies, USA) to 10 and 20 ng/μL final concentrations and stored at 4 °C or at −20 °C for long-term storage.

Primers and Probes. Primers and probes were purchased from Biolegio (Nijmegen, The Netherlands) or Eurogentec (Belgium). Table 1 shows the primers and probes used for the multitarget element-specific PCR plate. These were either taken from the literature

Table 1. Element-Specific Methods: Primers and Probes

no.	primer or probe[a] name	sequence 5′−3′	product length (bp)	ref
1	FatA primer1	GGTCTCTCAGCAAGTGGGTGAT	76	1
	FatA primer2	TCGTCCCGAACTTCATCTGTAA		1
	FatA probe	*ATGAACCAAGACACAAGGCGGCTTCA*		1
2	ZM1-F	TTGGACTAGAAATCTCGTGCTGA	79	1
	ZM1-R	GCTACATAGGGAGCCTTGTCCT		1
	probe ZM	*CAATCCACACAAACGCACGCGTA*		1
3	SPS-f	TTGCGCCTGAACGGATAT	81	4
	SPS-r	CGGTTGATCTTTTCGGGATG		4
	SPS-P	*GACGCACGGACGGCTCGGA*		4
4	Lec F	CCAGCTTCGCCGCTTCCTTC	93	1
	Lec R	GAAGGCAAGCCCATCTGCAAGCC		1
	Lec P	*CTTCACCTTCTATGCCCCTGACAC*		1
5	GluA3-F	GACCTCCATATTACTGAAAGGAAG	121	1
	GluA3-R	GAGTAATTGCTCCATCCTGTTCA		1
	GluD1 probe	*CTACGAAGTTTAAAGTATGTGCCGCTC*		1
6	UGP-af7	GGACATGTGAAGAGACGGAGC	88	1
	UGP-ar8	CCTACCTCTACCCCTCCGC		1
	UGP-sf1 probe	*CTACCACCATTACCTCGCACCTCCTCA*		1
7	wx012-5′	GTCGCGGGAACAGAGGTGT	102	5
	wx012-3′	GGTGTTCCTCCATTGCGAAA		5
	wx012-T	*CAAGGCGGCCGAAATAAGTTGCC*		5
8	acp1 primer 1	ATTGTGATGGGACTTGAGGAAGA	76	1
	acp1 primer 2	CTTGAACAGTTGTGATGGATTGTG		1
	acp1 probe	*ATTGTCCTCTTCCACCGTGATTCCGAA*		1
9	P35S-1-5′	ATT GAT GTG ATA TCT CCA CTG ACG T	101	6, 7
	P35S-1-3′	CCT CTC CAA ATG AAA TGA ACT TCC T		6, 7
	P35S-Taq	*CCC ACT ATC CTT CGC AAG ACC CTT CCT*		6, 7
10	NOS ter 2-5′	GTC TTG CGA TGA TTA TCA TAT AAT TTC TG	153	6, 7
	NOS ter 2-3′	CGC TAT ATT TTG TTT TCT ATC GCG T		6, 7
	NOS-Taq	*FAM-AGA TGG GTT TTT ATG ATT AGA GTC CCG CAA-TAMRA*		6, 7
11	PFMV 1-5′	ATCAACAAGGTACGAGCCATATC	120	8
	PFMV 1-3′	TGAGGCTTTGGACTGAGAATTC		8
	PFMV-1-Taq	*CCAAGAAGGAACTCCCATCCTCAAAGGTTT*		8
12	epsps 1-5′	GCCTCGTGTCGGAAAACCCT	118	9
	epsps 3-3′	TTCGTATCGGAGAGTTCGATCTTC		9
	epsps-probe	*TGCCACGATGATCGCCACGAGCTTCC*		this publication, AB209952.1
13	epsps2 1-5′	GTCTCGTTTCTGAAAACCCTGT	118	8
	epsps2 1-3′	TTAGTGTCGGAGAGTTCGATCTTAG		8
	epsps2-1-Taq	*TGATCGCTACTAGCTTCCCAGAGTTCATGG*		8
14	cry1A 4-5′	GGACAACAACCCMAACATCAAC	152	9
	cry1A 4-3′	GCACGAACTCGCTSAGCAG		9
	Cry1A(b)-probe	*CATCCCGTACAACTGCCTCAGCAACCCTG*		this publication, AY326434
15	Cry1Ac-F(/R)-n4	TTCAGGACCAGGATTCAC	165	this publication, EU880444.1
	Cry1AcR-n2	GTGAATAGGGGTCACAGAAGCATA		10
	Cry1AcP-n3	*TCTGGTAGATGTGGATGGGAAGT*		10

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 1. continued

no.	primer or probe[a] name	sequence 5′−3′	product length (bp)	ref
16	Cry3Bbf-n2	CCGCCCAGGACTCCATCG	231	this publication, http://gmdd.shgmo.org/sequence/view/18
	Cry3Bbr-n2	GAGGCACCCGAGGACAGG		this publication, http://gmdd.shgmo.org/sequence/view/18
	Cry3BbP-n3	*CTGCCGCCTGAGACCACTGACGAGC*		this publication, http://gmdd.shgmo.org/sequence/view/18
17	Cry1 Fr-n1	GACGTGGATCCTCATCTGCAATC	95	this publication, M73254
	Cry1 Fr-n2	GCAACACGGCTGGCAATCG		this publication, http://gmdd.shgmo.org/sequence/view/14
	Cry1Fp-n1	*CGCCACCAGGATTGAAGACCCCGTAAC*		this publication, M73254
18	Patf-n2	GACAGAGCCACAAACACCACAA	144	10
	Patr-n2	CAATCGTAAGCGTTCCTAGCCT		10
	Patp-n2	*GCCACAACACCCTCAACCTCA*		10
19	BAR 2-5′	ACTGGGCTCCACGCTCTACA	186	8
	BAR 2-3′	AAACCCACGTCATGCCAGTTC		8
	BAR-1-Taq	*CATGCTGCGGGCGGCCGGCTTCAAGCACGG*		8
20	npt 1-5′	GACAGGTCGGTCTTGACAAAAAG	155	9
	npt 1-3′	GAACAAGATGGATTGCACGC		9
	nptII-probe	*TGCCCAGTCATAGCCGAATAGCCTCTCCA*		this publication, HM066935
21	AINT 2-5′	TCGTCAGGCTTAGATGTGCTAGA	112	8
	AINT 2-3′	CTGCATTTGTCACAAATCATGAA		8
	AINT-2-Taq	*TTTGTGGGTAGAATTTGAATCCCTCAGC*		8
22	B'nase-F-n4	TGGTACCGGTTATTCAACACG	192	this publication, DI041986
	BNaseR-n3	GAGGGCTTGTGCTTCTGATTTT		10
	BNaseP-n3	*GTCTGAAGATAATCCGCAACCC*		10
23	BstarF-n2	AACAAATCAGAAGTATCAGCGACCT	145	10
	BstarR-n2	AACTGCCTCCATTCCAAAACG		10
	BStarP-n3	*ACCTGGACGCTTTATGGGATT*		10
24	CaMV-forward	GGCCATTACGCCAACGAAT	89	11
	CaMV-reverse	ATGGGCTGGAGACCCAATTTT		11
	CaMV-probe	*TTCTCCGAGCTTTGTC*		11

[a]All probes: 5′ 6-FAM and 3′ TAMRA.

(*FatA*, *hmg*, *Le1*, *GS*, *UGP*, *acp(1)*;[1] *SPS*;[4] *Wx-D01*;[5] *35S* promoter, *nos* terminator;[6,7] *pFMV*, *cp4-epsps*(2), *BAR*, *rActin1*,[8] *cp4-epsps*(1) except probe, *Cry1A(b)* except probe, *nptII* except probe;[9] *Cry1Ac* except forward primer, *PAT*, *barnase* except forward primer, *barstar*;[10] CaMV[11]) or designed with Beacon Designer 7.0 (Premier Biosoft, Palo Alto, CA, USA) (*cp4-epsps*(1) probe, *Cry1A(b)* probe, *Cry1Ac* forward primer, *Cry3Bb1* primers and probe, *Cry1F* primers and probe, *nptII* probe, *barnase* forward primer).

Real-Time PCR Reactions. Real-time PCRs were performed on BioRad i-Cyclers iQ and MyiQ with Optical System software version 3.1 or iQ5 Optical systems software version 2 (RIKILT) and Applied Biosystem 7300 Real-Time PCR system (NVWA). All reaction volumes were at 25 μL, and for all methods the Diagenode master mix (Diagenode, Belgium) (RIKILT) or Eurogentec master mix (NVWA) was used. Event-specific methods were carried out as described before.[12] All forward and reverse primers were used at a concentration of 400 nM, all probe concentrations were at 200 nM, and 50 ng of DNA was used per reaction. The PCR program was as follows: decontamination UNG (uracil-DNA glycosylase) 120 s at 50 °C, initial denaturation 600 s at 95 °C, amplification 45 cycles of 15 s at 95 °C and 60 s at 60 °C. "Ready-to-use" plates were provided by the European Reference Laboratory for GM Food and Feed.[13,14] The plates were used according to the manufacturer's instuctions.[15]

Specificity. The specificity of the primer and probe sets was evaluated experimentally by performing element-specific PCRs (50 ng/reaction) on the reference materials listed in Table 2. The reference materials were obtained from IRMM (Geel, Belgium) or AOCS (Champaign, IL, USA). Known trace contaminations of the reference materials with other GMOs (data not shown, Table 2) were taken into account when scoring Table 3 was made. This table was used to analyze the element screening results obtained for the sample analyses.

Detection Limit, PCR Efficiencies, Squared Coefficient of Correlation, R^2. For all methods applied it was verified whether the methods were capable of detecting at least 0.1% GMO (w/w) or 25 genome copies with 95% reliability to ensure that the LOD would be at least 0.1% (w/w).[13] This was verified in four to six PCR runs with eight repetitions (Supporting Information Table A). All methods applied met this criterion, except for the *barnase* PCR (68%) and the *acp(1)* cotton PCR (88%). The PCR efficiencies of almost all methods were >90%, except for *Wx-D01* (80%), *nos* terminator (88%), *Cry1A(b)* (88%), and Cry1F (82%). All R^2 values were >0.99 (Supporting Information Table B).

Table 2. Reference Materials Used for Element PCRs

reference material	code	% GMO	supplier	trace contamination
MS8 canola leaf DNA	AOCS 0306-F	100	AOCS	
RF3 canola leaf DNA	AOCS 0306-G	100	AOCS	
T45 canola leaf DNA	AOCS 0208-A	100	AOCS	
RT73/GT73 canola	AOCS 0304-B	>99.19	AOCS	
Bt11 maize	ERM BF412f	4.89	IRMM	
Bt176 maize	ERM BF411f	5.00	IRMM	
DAS-59122-7 maize	ERM BF424d	9.87	IRMM	
DAS-59122-8 maize	EU-RL	1	EU-RL	
event 3272 maize	ERM BF420c	9.8	IRMM	
GA21 maize	ERM BF414f	4.29	IRMM	MON810 maize
MIR604 maize	ERM BF423d	9.85	IRMM	
MON810 maize	ERM BF413f	5.00	IRMM	
MON863 maize	ERM BF416d	9.85	IRMM	
MON88017 maize	AOCS 0406-D	>99.05	AOCS	MON810 maize
NK603 maize	ERM BF415f	4.91	IRMM	
NK603 × MON863 maize	AOCS 0406-B	>99.05	AOCS	MON810 maize
NK603 × MON863 × MON810 maize	AOCS 0406-C	>89.85	AOCS	
TC1507 maize	ERM BF418d	9.86	IRMM	MON810 maize
T25 maize leaf DNA	AOCS 0306-H	100	AOCS	
LL62 rice DNA	AOCS 0306-I	100	AOCS	
DP305423 soy	ERM BF425d	10.00	IRMM	GTS 40-3-2 soy
DP356043 soy	ERM BF426d	10.00	IRMM	
MON89788 soy	AOCS 0906-B	>99.4	AOCS	GTS 40-3-2 soy
A5547-127 soy leaf DNA	AOCS 0707-C	100	AOCS	
A2704-12 soy leaf DNA	AOCS 0707-B	100	AOCS	
GTS 40-3-2 soy (Roundup Ready)	ERM BF410f	5.00	IRMM	
H7-1 sugar beet	ERM BF419b	100	IRMM	
EH92-527-1 potato	ERM BF421b	100	IRMM	
281-24-236 ×3006-210-23 cotton	ERM BF422d	10.00	IRMM	
MON1445 cotton	AOCS 0804-B	>99.4	AOCS	MON531 cotton
MON15985 cotton	AOCS 0804-D	>98.45	AOCS	
MON15985 × MON1445 cotton	AOCS 0804-F	>99.4	AOCS	
MON531 cotton	AOCS 0804-C	>97.39	AOCS	
MON531 × MON1445 cotton	AOCS 0804-E	>99.05	AOCS	
wheat	biological wheat	0	supermarket	

RESULTS AND DISCUSSION

Results of Element Screening on 50 Feed Samples. Fifty animal feed samples, almost all labeled as GMO, were screened with the extended element screening. The results are shown in Table 4. With this approach all samples were screened for the presence of soy, maize, canola, potato, wheat, sugar beet, and cotton, regardless of the ingredient declaration. As a result, crops were detected that were not claimed as ingredients in several samples. This may be botanical contamination, but as this was not the focus of this study this was not investigated further. Most samples were positive for *35S* promoter, *nos* terminator, and *cp4-epsps*. No clear indications for *Cry*-containing GMOs were found. In some cases only one of the duplicate PCR reactions for an element was positive. From three samples no clear positive results were obtained (samples 11, 47, and 50). This could be explained by insufficient DNA quality or by the absence of all targets for the element screening methods on the plate. Those three samples were not investigated further in this study. The results of the element screening were compared with Table 3, comprising the specificity of the element methods on different GMO events. From this comparison, a list was made of GMO events that were potentially present in the samples (Table 5). From the list in Table 5, samples were selected for a targeted approach to confirm the potential presence of GMO events (Table 6). Because the goal of this study was to evaluate the practical easiness of the element screening plate, not all theoretically possible EU-authorized events were checked in these GMO-labeled samples.

Additional Tests Based on Positive Cp4-epsps(1). In 41 samples a positive *cp4-epsps*(1) was observed in two of two PCR reactions, and in 6 samples *cp4-epsps*(1) was detected in one of two PCR reactions (Table 4). In all these 47 samples also the *35S* promoter and *nos*-terminator were detected, and therefore these 47 samples most likely contained GTS 40-3-2 soy. This assumption was checked and confirmed in 12 samples (Table 6). One sample (sample 8) was labeled as non-GMO soy. In this sample the percentage of GTS 40-3-2 soy found was too low for quantification. As the rest of the samples analyzed were all labeled as containing GMOs, the presence of GTS 40-3-2 soy was not an infringement of European GMO regulations. In the soy-containing samples also DP305423 and DP356043 events were checked. Both of these soy varieties are EU-unauthorized and contain neither the *35S* promoter nor the *nos*-terminator nor any other elements present on the screening plate. All samples tested with DP305423 and DP356043 event methods were negative (results not shown).

Additional Tests Based on Positive Cp4-epsps(2). Four mixed feed samples (samples 14, 36, 46, and 48) were found

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 3. Scoring Table for the Element Screening Results

Crop	Event	*35S promoter*	*Nos terminator*	*pFMV*	*cp4-epsps(1)*	*CP4epsps(2)*	*Cry1A(b)*	*Cry1Ac*	*Cry3Bb1*	*Cry1F*	*PAT*	*BAR*	*nptII*	*r-Actin1*	*Barnase*	*Barstar*	*CaMV*
canola	MS8		■									■			■		
	RF3		■									■				■	
	T45	■									■						
	RT73/GT73			■		■											
maize	Bt11	■	■				X				■						
	Bt176	■					■					■					
	DAS-59122-7	■									■						
	DAS-59132-8 (E32)	■									■						
	Event 3272		■														
	GA21		■											■			
	MIR604		■														
	MON810	■					■										
	MON863	■	■						■				■	■			
	MON88017	■	■		■	▒			■					■			
	NK603	■	■		■	▒								■			
	NK603*MON863	■	■		■	▒			■				■	■			
	NK603*MON863*MON810	■	■		■	▒	■		■				■	■			
	TC1507	■								■	■						
	T25 maize	■									■						
rice	LL62 rice	■										■		■			
soy	MON89788					■											
	A5547-127	■									■						
	A2704-12	■									■						
	DP305423																
	DP356043																
	GTS 40-3-2	■	■		■	▒											
sugar beet	H7-1			■		■											
potato	EH92-527-1		■										■				
cotton	281-24-236*3006-210-23							X		■	■						
	MON1445	■	■	■		■							■				
	MON15985	■	■					■					■				
	MON15985*MON1445	■	■	■		■		■					■				
	MON531	■	■					■					■				
	MON531*MON1445	■	■	■		■		■					■				

■ detected, result expected theoretically
▒ detected at high Ct, result not expected theoretically
X not detected, contains element with sequence differences
□ not detected, result expected theoretically

positive for soy, canola, *cp4-epsps*(1), and *cp4-epsps*(2). From the small difference in Ct between *cp4-epsps*(1) and *cp4-epsps*(2) it was expected that another GMO would be present in addition to GTS 40-3-2 soy, and the samples were tested for MON89788 soy and RT73 canola events (Table 6). In two samples (samples 14 and 44) MON89788 soy was found, and in one sample (sample 48) RT73 canola was detected. In sample 48 the canola and *cp4-epsps*(2) methods gave signals at high Ct values. Although the *pFMV* PCR was negative, the sample was checked with the RT73 canola event method because of the positive *cp4-epsps*(2) as explained above. A low amount of RT73 was detected (three of four reactions were positive with high Ct) (Table 6). This demonstrates that indeed at high Ct values not all element methods may be positive.

Additional Tests Based on Positive PAT. In seven mixed feed samples the *PAT* element was detected (samples 13, 14, 16, 17, 18, 20, and 44). These samples were tested for eight events containing the *PAT* gene:[14] T45 canola, Bt11 maize, DAS59122-7 and DAS59132−8 maize, T25 maize, TC1507 maize, A2704-12 soy, and A5547-127 soy. In all seven samples A2704-12 soy was detected (Table 6). A2704-12 soy contains the *35S* promoter and the *PAT* element. In three samples TC1507 maize and in two samples DAS59122-7 maize were also detected (Table 6). In sample 16 the TC1507 event (which contains the *PAT* and *Cry1F* elements) was detected in three of four PCR reactions, although the *Cry1F* element was not detected in the element screening plate. In sample 20 the *Cry1F* element was detected in one of the two PCR reactions, and this was confirmed by a positive TC1507 event-specific method. These results indicate that adding the *PAT* element to routine screening will be useful.

Inconclusive Results for the nptII Element. The *nptII* element method gave a positive signal in 12 samples, of which 9 times in one of two PCR reactions. In the three other samples (samples 10, 40, and 43) the *nptII* element was detected in two of two PCR reactions. In samples 10 and 40 the *nptII* element could be theoretically explained by the presence of MON863 maize presuming the *Cry3Bb1* element was not detected due to differences in sensitivity of the element methods. This was checked by analyzing these samples with a MON863 event-specific method. No MON863 maize event was detected. In sample 43 the positive *nptII* element PCR could not be theoretically explained by a known GMO event method. Also, sample 43 was tested for MON863 event, and it was found negative. The frequent *nptII* signals could result from bacteria

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 4. PCR Results of the Element Screening on 50 Real-Life Feed Samples

sample no.	samplename	canola		maize		rice		soy		sugar beet		potato		wheat		cotton		35S promoter		Nos-terminator		pFMV		cp4-epsps (1)		cp4-epsps (2)		Cry1A(b)		Cry1-Ac		Cry3-Bb1		Cry1-F		PAT		BAR		nptII		rActin1		barnase		barstar		CaMV	
1	palmseed meal	38	-	39	-	-	-	37	37	-	-	-	-	-	-	-	-	39	40	42	-	-	-	41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	soybean meal	39	39	39	37	-	43	28	28	-	-	-	-	-	-	-	-	27	29	29	29	-	-	29	29	-	38	-	-	-	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-	-	-	-	-
3	palmseed meal	39	37	-	-	-	-	37	37	-	-	-	-	-	-	-	-	38	37	40	39	-	-	37	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	citrus pulp	-	-	-	-	-	-	37	37	-	-	-	-	-	-	-	-	38	40	-	-	-	-		39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	mixed feed	28	28	-	28	-	-	27	28	-	-	31	32	37	35	-	-	28	28	29	29	-	-	29	29	38	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6	horse feed	31	31	33	33	-	-	27	28	-	-	37	36	32	33	-	-	27	27	29	29	-	-	30	30	40	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	-	-	-	-	-	-
7	soybean meal	-	-	-	37	-	-	27	25	-	-	37	38	-	41	-	-	26	25	27	26	-	-	27	26	34	33	-	-	-	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	-	-	-
8	soybean meal non-GMO	-	-	-	37	-	-	25	25	-	-	-	-	-	-	-	-	38	35	-	37	-	-	37	37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	maize	36	37	22	23	-	-	35	36	-	-	-	-	36	38	-	-	34	35	38	36	-	-	35	36	-	-	-	-	-	-	-	-	-	-	39	-	-	-	-	-	-	-	-	-	-	-	-
10	barley	30	30	37	36	-	-	34	31	-	-	-	-	37	39	-	-	38	33	36	34	-	-	35	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	37	-	-	-	-	-	-	-
11	sunflower pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	pig feed	28	29	33	34	42	37	29	30	-	-	30	31	34	33	-	-	28	29	28	29	-	-	30	30	37	38	-	-	-	-	-	-	-	-	-	-	-	-	-	42	38	-	-	-	-	-	-
13	chicken feed	30	30	27	27	-	-	26	25	-	-	38	-	32	33	-	-	26	25	26	26	41	-	27	28	33	34	-	-	-	-	-	-	-	-	39	39	-	-	-	-	-	-	-	-	-	-	-	-
14	mixed feed	29	30	28	28	-	-	27	27	-	39	-	-	39	39	-	-	27	26	28	28	-	-	32	30	33	33	-	-	-	-	-	-	-	-	36	36	-	-	-	-	-	-	-	-	-	-	-	-
15	chicken feed	28	27	29	28	39	38	27	26	-	-	38	-	37	34	-	-	27	26	28	26	-	-	28	26	35	34	-	-	-	-	-	-	-	-			-	-	-	-	34	34	-	-	-	-	-	-
16	mixed feed	37	35	29	29	36	35	28	27	-	-	-	-	36	35	-	-	28	27	29	28	-	-	28	28	33	31	-	-	-	-	-	-	-	-	37	35	-	-	-	-	32	31	-	-	-	-	-	-
17	mixed feed	35	37	29	29	35	36	27	27	-	-	-	-	35	36	-	-	27	27	27	28	-	-	27	28	32	32	-	-	-	-	-	-	-	-	37	37	-	-	-	-	31	31	-	-	-	-	-	-
18	mixed feed	35	36	30	30	36	35	28	28	-	-	-	-	39	37	-	-	28	28	29	29	-	-	32	29	34	34	-	40	-	-	-	-	-	-	38	40	-	-	-	-	32	31	-	-	-	-	-	-
19	mixed feed	32	33	26	26	-	-	26	25	-	-	33	32	32	32	-	-	25	24	25	25	-	-	25	25	35	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	-	-	-	-	-	-
20	horse feed	29	29	27	28	-	43	27	27	-	-	-	-	32	34	-	-	27	27	27	28	-	-	29	29	33	33	-	-	-	-	-	-	40		38	37	-	-	39	-	40	39	-	-	-	-	-	-
21	soybean meal	-	40	-	37	-	38	26	26	-	-	-	-	39	39	-	-	26	25	26	26	-	-	26	26	35	34	-	-	-	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	-	-	-
22	mixed feed	37	-	35	35	-	-	38	36	-	-	-	-	30	29	-	-	38	36	37	35	-	-	37	37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	37	-	-	-	-	-	-	-	-
23	mixed feed	37	37	29	29	-	-	28	28	-	-	33	33	37	36	-	-	28	28	29	28	-	-	28	28	-	40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	chicken feed	35	35	30	30	-	-	30	30	-	-	33	33	37	35	-	-	38	38	38	39	-	-	38	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25	wheat bran feed	-	39	-	40	-	-	39	38	-	-	-	-	41	36	-	-	38	38	40	41	-	-	43		32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
26	pig feed	39	38	36	37	-	-	27	27	-	-	-	-	-	41	-	-	26	27	28	28	-	-	28	28	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
27	soybean meal	40	39	36	-	-	-	28	28	-	-	38	37		40	-	-	27	28	29	28	-	-	28	28	40	41	43	42	-	-	-	-	-	-	-	-	-	-	-	38	-	-	-	-	-	-	-	-
28	soybean meal	-	-	42	38	-	-	27	27	-	-	-	-	38	39	-	-	26	26	27	27	-	-	27	27	39	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-	-	-
29	chicken feed	35	35	28	29	-	-	28	29	-	-	18	22	35	35	-	-	37	35	38	37	-	-	38	37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	broken rice	-	-	-	-	31	32	36	36	-	-	-	-	-	-	-	-	36	36	36	36	-	-	35	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	41	28	28	-	-	-	-	-

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 4. continued

sample no.	samplename	canola		maize		rice		soy		sugar beet		potato		wheat		cotton		35S promoter		Nos-terminator		pFMV		cp4-epsps (1)		cp4-epsps (2)		Cry1A (b)		Cry1-Ac		Cry3-Bb1		Cry1-F		PAT		BAR		nptII		rActin1		barnase		barstar		CaMV	
31	soybean meal	-	-	-	-	-	42	26	26	-	-	39	-	-	-	-	-	25	25	26	26	-	39	26	26	37	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	-	-	-	-	-	-	-
32	soybean meal	-	-	-	-	40	-	27	27	-	-	-	-	-	-	-	-	38	38	40		-	-	39	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-	-	-
33	soybean meal	-	-	-	37	-	-	28	28	-	-	-	-	-	-	-	-	40		41	-	-	-	38		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-	-	-
34	citrus pulp	-	-	-	-	-	-	38	37	-	-	39	-	-	-	-	-	40	40	-	-	-	-	39		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35	soybean meal	-	-	-	38	-	-	26	27	-	-	-	-	-	-	-	-	30	28	35	37	-	-	32	32	35	36	42	-	-	-	-	-	-	-	-	-	-	-	35	-	39	43	-	-	-	-	-	-
36	mixed feed	28	28	34	36	39	39	29	29	-	-	-	-	-	-	-	-	32	31	43	-	-	-	36	39	40	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	35	36	-	-	-	-	-
37	mixed feed	28	29	30	31	42	-	30	30	-	-	-	-	43	42	-	-	30	32	43	43	-	-	39		39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	40	-	-	-	-	-
38	mixed feed	28	27	30	30	42	-	30	30	-	-	-	-	33	33	-	-	30	30	31	30	-	-	30	30	39	38	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
39	soybean meal	-	-	-	-	-	41	27	26	-	-	41	42	40	42	-	-	26	26	27	26	-	-	26	26	34	34	-	-	-	-	-	-	-	-	-	-	-	-	34	-	38	38	-	-	-	-	-
40	pig feed	29	28	38	35	-	-	28	27	-	-	-	-	33	30	-	-	28	26	29	27	-	38	30	26	38	34	-	-	-	-	-	-	-	-	-	-	-	-	42	36	-	38	-	-	-	-	-
41	horse feed	31	31	31	30	-	-	28	28	39	-	35	36	34	32	-	-	28	28	30	29	-	-	30	28	38	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	-	-	-	-	-
42	soybean meal	36	34	39	38	-	42	28	27	-	-	-	-	41	39	-	-	27	26	28	27	-	-	28	27	37	42	-	-	-	-	-	-	-	-	-	-	-	-	33	-	-	38	-	-	-	-	-
43	soybean meal	42	-	-	-	42	-	27	27	-	-	-	-	-	-	-	-	26	26	27	27	-	-	27	27	35	36	-	-	-	-	-	-	-	-	-	-	-	-	37	37	-	-	-	-	-	-	-
44	mixed feed	32	31	28	28	-	43	27	26	-	-	38	32	33	32	-	-	27	26	27	27	-	-	27	27	42	34	-	-	-	-	-	-	-	-	38	37	-	-	38	-	37	39	-	-	-	-	-
45	mixed feed	32	32	32	32	-	-	31	31	-	-	37	-	39	41	-	-	30	30	33	33	-	-	39	39	41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
46	horse feed	31	31	31	31	-	-	34	34	-	-	32	34	40	40	-	-	35	34	36	36	-	-	40	37	35	37	-	-	-	-	-	-	-	-	42	-	-	-	-	-	-	-	-	-	-	-	-
47	beet pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
48	horse feed	33	33	35	35	-	-	31	32	39	40	-	-	37	43	-	-	31	31	33	33	-	-	35	39	38	42	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
49	horse feed	30	31	27	27	-	-	28	28	-	-	-	-	34	32	-	-	28	27	29	29	40	-	30	29	39	36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	36	-	-	-	-
50	mixed feed	41	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	39	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 5. Possibly Present GMOs Based on the Element Screening Results (Table 4) in Combination with Specificity of the Element Methods (Table 3)[a]

		canola				maize												rice			soy			
sample no.	sample name	MS8	RF3	T45	RT73/ GT73	Bt11	Bt176	CBH351	DAS-59122-7	**DAS-59132**	GA21	MON810	MON863	MON88017	NK603	TC1507	T25	**Bt63**	**LL601**	**LL62**	MON89788	**A5547-127**	A2704-12	GTS 40-3-2
1	palmseed meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
2	soybean meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		X
3	palmseed meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		X
4	citrus pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		X
5	mied feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		X
6	horse feed	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	-	-	-	X
7	soybean meal	-		-	-	X	-	-	X	X	-	-	-	-	-	-	X	-	-	-	X	X	X	X
8	soybean meal non-GMO	-		-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
9	maize	-	-	X	-	X	-	-	X	X	-	-	-	-	-	-	X	-	-	-	-	X	X	X
10	barley	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
11	sunflower pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	pig feed	-	-	-	-	-	-	-	-	-	-	-	X	-	X	-	-	-	-	-	X	-	-	X
13	chicken feed	-	-	-	X	X	-	-	X	X	-	-	-	-	-	-	X	-	-	-	X	X	X	X
14	mied feed	-	-	-	-	X	-	-	X	X	-	-	-	-	-	-	X	-	-	-	X	X	X	X
15	chicken feed	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	X	-	-	X
16	mied feed	-	-	X	-	X	-	-	-	-	X	-	-	-	-	-	X	-	-	-	-	X	X	X
17	mied feed	-	-	X	-	X	-	-	X	X	X	-	-	-	-	-	X	-	-	-	X	X	X	X
18	mied feed	-	-	X	-	X	-	-	X	X	X	-	-	-	-	-	X	-	-	-	X	X	X	X
19	mied feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
20	horse feed	-	-	X	-	X	-	-	X	X	X	-	-	-	X	X	X	-	-	-	X	X	X	X
21	soybean meal	-	-	X	-	X	-	-	X	X	-	-	-	-	-	X	-	-	-	-	X	X	X	X
22	mied feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
23	mied feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
24	chicken feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	X
25	wheat bran feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	X
26	pig feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
27	soybean meal	-	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	X
28	soybean meal	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	X	-	-	X

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Table 5. continued

		canola				maize												rice			soy			
sample no.	sample name	MS8	RF3	T45	RT73/ GT73	Bt11	Bt176	CBH351	DAS-59122-7	**DAS-59132**	GA21	MON810	MON863	MON88017	NK603	TC1507	T25	**Bt63**	**LL601**	**LL62**	MON89788	**A5547-127**	A2704-12	GTS 40-3-2
29	chicken feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
30	broken rice	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
31	soybean meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
32	soybean meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
33	soybean meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
34	citrus pulp	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
35	soybean meal										X	X	-	-	X	-	-	-	-	-	X	-	-	X
36	mixed feed	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	X	-	-	X
37	mied feed	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	X	-	-	X
38	mixed feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
39	soybean meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
40	pig feed	-	-	-	X	-	-	-	-	-	X	-	X	-	X	-	-	-	-	-	X	-	-	X
41	horse feed	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-	-	-	-	-	X	-	-	X
42	soybean meal	-	-	-	-	-	-	-	-	-	X	-	X	-	X	-	-	-	-	-	X	-	-	X
43	soybean meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
44	mixed feed	-	-	X	-	X	-	-	X	X	X	-	X	-	X	-	X	-	-	-	X	X	X	X
45	mixed feed	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	X	X	-	X
46	horse feed	-	-	X	-	X	-	-	X	X	-	-	-	-	-	-	X	-	-	-	X	X	X	X
47	beet pulp	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	X	-	-
48	horse feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
49	horse feed	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	X
50	mixed feed	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[a]X, possible GMO; -, not possible GMO; boldface, at time of the study EU unauthorized GMO.

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 6. Theoretically Possible GMOs Based on Element Screening Results and Confirmed GMOs of Some Selected Samples[a]

		canola		maize									soy			
sample no.	sample name	T45	RT73	Bt11	DAS-59122-7	**DAS-59132**	GA21	MON810	MON863	NK603	TC1507	T25	MON 89788	**A5547-127**	A2704-12	GTS 40-3-2
8	soybean meal non-GMO	-	-	-	-	-	-	-	-	-	-	-	-	-	-	***X***
13	chicken feed	-	*X*	*X*	*X*	*X*	-	-	-	-	-	*X*	*X*	*X*	***X***	***X***
14	mixed feed	-	-	*X*	*X*	*X*	-	-	-	-	-	*X*	***X***	*X*	***X***	***X***
16	mixed feed	*X*	-	*X*	-	-	*X*	-	-	-	(*X*)	*X*		*X*	***X***	***X***
17	mixed feed	*X*	-	*X*	***X***	*X*	*X*	-	-	-	-	*X*	*X*	*X*	***X***	***X***
18	mixed feed	*X*	-	*X*	*X*	*X*	*X*	-	-	-	-	*X*	*X*	*X*	***X***	***X***
20	horse feed	*X*	-	*X*	***X***	*X*	*X*	-	-	*X*	***X***	*X*	*X*	*X*	***X***	***X***
35	soybean meal	-	-	-	-	-	*X*	*X*	-	*X*	-	-	*X*	-	-	***X***
36	mixed feed	-	-	-	-	-	*X*	-	-	*X*	-	-	*X*	-	-	***X***
44	mixed feed	*X*	-	*X*	*X*	*X*	*X*	-	*X*	*X*	(*X*)	*X*	*X*	*X*	***X***	***X***
46	horse feed	*X*	-	*X*	*X*	*X*	-	-	-	-	-	*X*	***X***	*X*	*X*	***X***
48	horse feed	-	(*X*)	-	-	-	-	-	-	-	-	-	*X*	-		***X***

[a] *X*, possible GMO; -, not possible GMO; boldface *X*, possible GMO that was confirmed with event specific method; (*X*), confirmed with event-specific method and theoretically possible, when not all elements of the GMO were detected in the element screening due to low copy numbers; boldface GMO name, at time of the study EU unauthorized GMO.

present in the samples. This would mean that the *nptII* element would not be very useful for GMO screening. This is confirmed by other data that indicate a large number of false positives in the case of *nptII* (results not shown).

Results of the Interlaboratory Study. To further investigate the robustness of the extended element screening in practical sample analyses, eight samples were analyzed in a concise interlaboratory study. These eight samples were analyzed in two different laboratories using the same PCR conditions but with different (validated and accredited) methods for DNA isolation and with different brands of chemicals and PCR machines. The goal of the study was to test if there would be significant differences in the outcome of the screenings. This may occur when the PCR methods are transferred to another laboratory that combines the PCR methods with a different DNA isolation method and uses a different brand of machine and different brands of chemicals. Seven feed samples and one food sample (cheese snack) that were investigated earlier using event-specific methods were tested with the element screening plate: 1, young horse kernel, GTS 40-3-2 soy, 76%; 2, maize meal, GTS 40-3-2 soy, 91%; 3, tropical seed, NK603 detected, MON810 detected, Bt11 14%, GA21 4.4%, GTS 40-3-2 soy detected; 4, maize meal, Bt11 14.5%, NK603 1%, GA21 0.1%, TC1507 0.2%, T25 detected, Bt176 detected, MON810 maize 32.3%, GTS 40-3-2 soy 75%; 5, cheese snack, MON810, MON863, NK603, TC1507 detected; 6, maize meal, GTS 40-3-2 soy 103%; 7, bird feed, MS8 canola detected, Rf3 canola detected, RT73 canola 19.1%; 8, maize gluten, GTS 40-3-2 soy detected. The results are shown in Table 7, including average Ct values, although these Ct values are of limited significance. This is because Ct values are dependent on the brand of machine and on the software settings for the baseline and threshold. As a result, the Ct values within different runs in one laboratory and between different laboratories cannot be directly compared. The Ct values are mainly shown to give an idea of the level of GMO content.

Most screening results could be explained by the GMO events that were detected with event-specific methods and in some cases also quantified. These results have been marked in boldface in Table 7. In sample 3 also the *BAR*, *barstar*, and *barnase* elements were detected. MS3, RF3, MS1, RF1, and RF2 canola events would explain the positive signals for these elements, but these methods were all negative when this sample was tested with the "ready-to-use" plate.[15] Although in sample 3 the *pFMV* element method was positive in one of two reactions in laboratory 2 (and twice negative in laboratory 1), no RT73 canola event was detected. Lower practical detection limits of the canola event methods in this particular sample can explain the positive signals for the element methods and the negative signals for the canola event methods. The late signals for *cp4-epsps*(2) were expected from the results of the specificity testing (Table 3) and were caused by the fact that this method slightly detects *cp4-epsps*(1). In samples 2, 3, 7, and 8 the signals for the *nptII* element (present in MON863 maize) were not expected to be caused by the presence of the MON863 event, because no signals were obtained for the *cry3Bb1* or *rActin* method, unless the *cry3Bb1* and *rActin* methods were negative in this sample because the level of MON863 event was very low. In sample 7, which gave signals for *nptII* in both laboratories, this was verified by carrying out the MON863 event method, but no MON863 event was detected. It is also possible that the *nptII* element was detected in bacterial DNA isolated from the sample. From the result of the robustness experiments it was concluded that overall there were no significant differences between the results from the two laboratories. At Ct values higher than 37 not always two of two PCR reactions were positive in both laboratories. This may be due to the low copy number of the target in combination with differences in detection limit for the element methods. For low amounts of GMO in a sample it can be more difficult to interpret the element screening results, and differences in detection limits of the element screening methods will have to be taken into account. However, if the screening comprises a large number of elements, the chances will increase that any unauthorized GMO present in the sample will be detected despite the relative differences in detection limits in the different matrices.

Conclusions. In this paper the application of an extended screening strategy for GMOs in real-life samples on the basis of GMO element-specific TaqMan real-time PCRs is described. The strategy was used in the first place to check these

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

Table 7. Results of Element Screening on Eight Samples in Two Different Laboratories[a]

		endogenous gene																							
sample no.[c]	lab no.	canola *FatA*	maize *HMG*	soy *Le1*	rice *SPS*	sugar beet *GS*	potato *UGP*	wheat *Wx-1*	cotton *acp1*	*35S* promoter	*Nos* terminator	*pFMV*	*cp4-epsps* (1)	*cp4-epsps* (2)	*Cry1A (b)*	*Cry1Ac*	*Cry3Bb1*	*Cry1F*	*PAT*	*BAR*	*nptII*	rice actin gene intron	*barstar*	*barnase*	CaMV
1	1	27	34	27	N	39	32	36	N	**27**	**28**	N	**27**	N	N	N	N	N	N	N	N	38	N	N	N
	2	29	35	31	N	41	34	38	N	**29**	**30**	N	**31**	N	N	N	N	N	N	N	N	N	N	N	N
2	1	35	25	30	N	38	38	37	N	**30**	**31**	N	**31**	N	N	N	N	N	N	N	39[b]	N	N	N	N
	2	37	25	32	N	39	38	38	N	**31**	**31**	N	**31**	41[b]	N	N	N	N	N	N	N	N	N	N	N
3	1	32	32	36	N	N	28	38	N	**32**	**34**	N	**34**	N	**40**[b]	N	N	N	**37**	39[b]	43[b]	**34**	N	N	N
	2	33	33	38	N	N	29	38	N	**32**	**32**	38[b]	**34**	40	**N**	N	N	N	**38**	38	N	**34**	37	39	N
4	1	30	25	31	38	N	34	37	N	**26**	**28**	N	**30**	N	**28**	N	N	N	**32**	N	N	**31**	N	N	N
	2	29	25	31	36	N	34	36	39	**26**	**28**	N	**31**	42	**30**	N	N	39[b]	**33**	N	N	**31**	N	N	N
5	1	38[b]	25	38	N	N	38[b]	N	N	**26**	**29**	N	**27**	N	**29**	N	**34**	**31**	**32**	N	**31**	**28**	N	N	N
	2	N	31	N	N	N	N	N	N	**32**	**33**	N	**33**	N	**38**	N	**39**	**38**	**41**	N	**37**	**34**	N	N	N
6	1	29	25	29	N	N	N	39	N	**30**	**32**	N	**30**	N	N	N	N	N	N	N	N	N	N	N	N
	2	31	26	32	N	N	N	39	N	**32**	**33**	N	**32**	N	N	N	N	N	N	N	N	N	N	N	N
7	1	26	38[b]	N	N	N	N	39	N	37[b]	**28**	**28**	N	**30**	N	N	N	N	**N**	**29**	38[b]	N	**29**	**32**	N
	2	27	38	39	N	N	N	37	N	**36**	**28**	**29**	N	**31**	N	N	N	N	**39**	**32**	30	N	**29**	**33**	N
8	1	34	27	33	N	N	37	42	N	**35**	**37**	N	**35**	N	39[b]	N	N	N	N	N	N	N	N	N	N
	2	36	26	34	N	N	39	41	N	**34**	**35**	N	**34**	N	N	N	N	N	N	N	37	N	N	N	N

[a]Ct values are averages of two PCRs; N, not detected. [b]Only one of two PCRs was positive. [c]Sample description and detected GMOs: 1, young horse kernel, GTS 40-3-2 soy, 76%; 2, maize meal, GTS 40-3-2 soy, 91%; 3, tropical seed, NK603 detected, MON810 detected, Bt11 14%, GA21 4.4%, GTS 40-3-2 soy detected; 4, maize meal, Bt11 14.5%, NK603 1%, GA21 0.1%, TC1507 0.2%, T25 detected, Bt176 detected, MON810 maize 32.3%, GTS 40-3-2 soy 75%; 5, cheese snack, MON810, MON863, NK603, TC1507 detected; 6, maize meal, GTS 40-3-2 soy 103%; 7, bird feed, MS8 canola detected, Rf3 canola detected, RT73 canola 19.1%; 8, maize gluten, GTS 40-3-2 soy detected. GMO screening results that were confirmed by event-specific methods are marked in boldface.

dx.doi.org/10.1021/jf4018146 | J. Agric. Food Chem. 2013, 61, 9097−9109

GMO-labeled feed samples for the potential presence of unknown unauthorized GMOs.

The multiple element screening plate proved to be a useful tool to obtain information on the sample composition of mixed food and feed. The presence of MON89788 soy in two samples would not have been detected if the more concise Waiblinger screening[3] had been used. In mixed samples containing multiple ingredients, for example, soy, maize, and canola, the strategy was effective as instead of around 30 individual event-specific methods, it was shown that a much lower number of relevant PCRs related to positive elements needed to be performed.

Furthermore, in all samples containing maize this strategy will in most cases lead to a significant reduction in the number of necessary confirmatory PCR analyses. Only in the case of single-ingredient GMO-labeled samples that are not maize samples it will be more effective to directly perform all available PCRs for the potential presence of unapproved GMOs. In this series no indications for unauthorized GMOs were found.

With increasing numbers of GMOs on the market, it has become more urgent to screen for (unknown) unauthorized GMOs. The element screening approach has the potential to give indications also for the presence of (unknown) unauthorized GMOs for which there are no event-specific methods available. This will require additional sequencing strategies that are currently in development, but not yet included in the present study. Adding more elements to the screening plate in the future will further increase the potential for detecting (unknown) unauthorized GMOs. Finally, it is expected that sequencing strategies will become of increasing importance in this field of research. The screening strategy as described in the present study will form an adequate basis for any future strategy to identify unauthorized GMOs in food and feed samples.

■ ASSOCIATED CONTENT

Supporting Information

Tables A and B. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This research was funded by the Dutch Ministry of Economic Affairs, Program 438: WOT02 Food Safety, Theme 4 Animal feed, projects "Detection and identification of genetically modified organisms" and "Food and Consumer Product Safety Authority − GMO detection in feed".

Notes

The authors declare no competing financial interest.

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