FOOD COMPOSITION AND ADDITIVES

Real-Time Detection of Genetically Modified Soya Using Lightcycler and ABI 7700 Platforms with TaqMan, Scorpion, and SYBR Green I Chemistries

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A comparative cross platform evaluation of real-time polymerase chain reaction detection of DNA sequences present in Roundup Ready soya was undertaken using the ABI 7700 and Roche Lightcycler detection systems in combination with 3 different detection chemistries: TaqMan, Scorpion primers, and SYBR Green I fluorescent dye. Various copy numbers of a plasmid containing the soya lectin sequence were used to determine the sensitivity and reproducibility of the different technology combinations and to examine both inter and intra machine variability. To examine the relative accuracy of each technology, the genetically modified soya content of baked products containing known amounts of Roundup Ready soya was determined by detection of lectin and the EPSPS transgene. It was determined that the combination of TaqMan detection chemistry and the ABI 7700 platform represented the best method for quantitative detection of genetically modified organisms in terms of both precision and accuracy.

The European Commission (EC) Novel Foods Regulation (258/97) came into force on May 15, 1997, and established a European Union (EU) wide system for the safety assessment and labeling of novel foods. Current regulation (49/2000) implemented (as amendment to 258/97) a 1% de minimis threshold for the adventitious contamination of nongenetically modified (GM) products from an identity preserved source. As a consequence, if 1% or less GM soya or maize is present, the product does not have to be labeled as containing a GM ingredient. To uphold these regulations, reliable and accurate quantitative methods for DNA determination must be available. Comparability of measurement is required but different testing laboratories use different analytical methods. The most predominant method used for quantitative polymerase chain reaction (PCR) is real-time analysis.

Real-time PCR allows continual monitoring of the accumulation of target DNA as each PCR cycle progresses. Fluorescent probes show an increase in fluorescence emission as a result of amplification, permitting product accumulation to be monitored against PCR cycle number. Thus, target DNA can be quantitatively measured. The 2 most widespread instrument platforms available to perform real-time PCR are the Lightcycler™ (Roche Diagnostics, Hertfordshire, UK) and the ABI 7700 Prism™ (Applied Biosystems, Foster City, CA). Although both allow measurement of the accumulation of target PCR products in real-time, they often use different chemistries. This study compared 3 such chemistries: TaqMan (1), Scorpion (2), and SYBR I Green (3–5) on each of the 2 real-time PCR detection platforms.

TaqMan probes are fluorogenic probes that hybridize within the target sequence amplified by conventional PCR primers. The 5′ end of the probe is labeled with a fluorescent reporter dye, the 3′ end with a quencher dye. The 5′ fluorescent label is cleaved from the quencher by the 5′ to 3′ exonuclease activity of the Taq DNA polymerase during PCR. The increase in fluorescence is therefore directly proportional to the amplification and accumulation of specific PCR products.

Scorpion primers are unimolecular specific probes held in a hairpin loop structure by a complementary stem sequence, while in the stem–loop structure, a quencher at the 3′ end of the stem–loop inhibits fluorescence from the fluorophore at the 5′ end. After amplification from the primer attached to the stem–loop structure, the specific probe sequence in the loop binds to its complement within the same DNA strand, opening the stem–loop structure. As a result, the fluorophore is no longer quenched and the fluorescent signal increases proportionally with PCR amplification. A PCR stopper sequence between the primer and the stem–loop prevents read-through of the stem–loop.

SYBR Green I is a nonspecific double-stranded DNA binding dye used to detect double-stranded DNA from 2 conventional PCR primers. Accumulation of PCR product is measured by an increase in fluorescence resulting from interaction of the dye with double-stranded DNA between the newly synthesized strands of DNA (3–5).

This study attempted to determine the comparability of quantitative PCR for GM food analysis using current different real-time technologies and chemistries. The precision and sensitivity of real-time detection was studied by using a lectin

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Table 1. PCR primer pairs and fluorescent probes

PCR assay	Primer name	Orientation	Sequence	Amplicon size	Reference
			TaqMan		
Endogenous PCR	Sltm1	Sense	5′-AACCGGTAGCGTTGCCAG-3′	80	
	Sltm2	Antisense	5′-AGCCCATCTGCAAGCCTTT-3′		8
	Sltmp	Sense probe	5′-tet-TTCGCCGCTTCCTTCAACTTCACCT-tam-3′		
EPSPS PCR	Sttmf3a	Sense	5′-GCAAATCCTCTGGCCTTTCC-3′	145	8
	Sttmr2a	Antisense	5′-CTTGCCCGTATTGATGACGTC-3′		
	Sttmpa	Sense probe	5′-fam-TTCATGTTCGGCGGTCTCGCG-tam3′		
			Scorpion		
Endogenous PCR	Lectin-Scor	Sense	5′-GCGGCCAACGCTACCGGTTTCTTTGTCCCAAATGG CCGC-MR-HG-GCCCTCTACTCCACCCCCATCC-3′	159	
	Lectin-Rev	Antisense	5′-GCCCATCTGCAAGCCTTTTTGTG-3′		
35S PCR	35s-Scor	Sense	5′-CCGCGGAAAATAAACATAGGGAACCCGCGG-MR-H G-GAGCCACCTTCCTTTTCCATTT-3′	170	
	35s-Rev	Antisense	5′-ACCCTTCAATTTAACCGATGCT-3′		
			SYBR Green I and Lectin Cloning PCR		
Endogenous PCR	Lectin-for	Sense	5′-GCCCTCTACTCCACCCCCATCC-3′	118	
	Lectin-rev	Antisense	5′-GCCCATCTGCAAGCCTTTTTGTG-3′		

gene sequence cloned into a plasmid. Different copy numbers of the plasmid were used to determine the sensitivity and reproducibility of detection with the different technologies and different probe systems. To examine the relative accuracy of the different technologies, the GM soya content of baked products containing known amounts of Roundup Ready soya was determined. The Roundup Ready gene was quantitated relative to the endogenous soya lectin gene and results were expressed as the percentage of GM soya relative to total soya content. Roundup Ready soya flour reference materials of known GM content were used to produce a calibration curve from which the concentration of GM soya in the baked products was elucidated.

Experimental

Sample and Standard Preparation

For construction of calibration curves, we used certified reference materials consisting of dried soya flour containing 0,

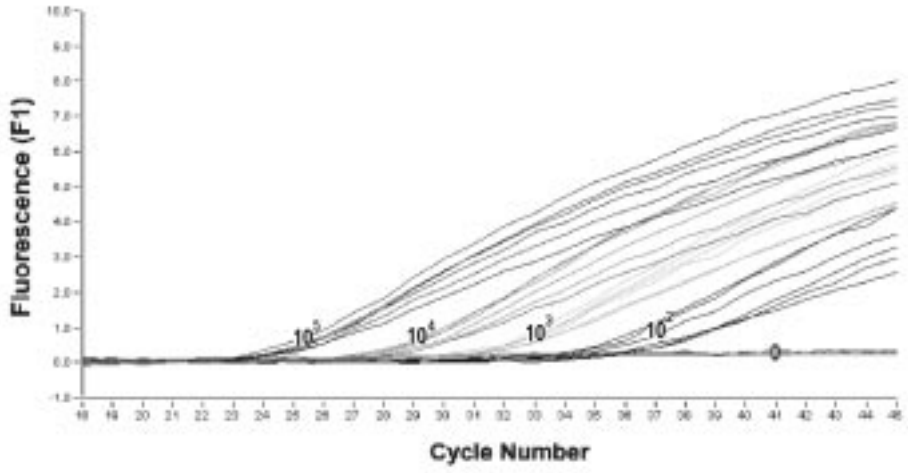

Figure 1. Lightcycler linear amplification plots for 6 replicates of 5 plasmid copy numbers using the Scorpion lectin primer system.

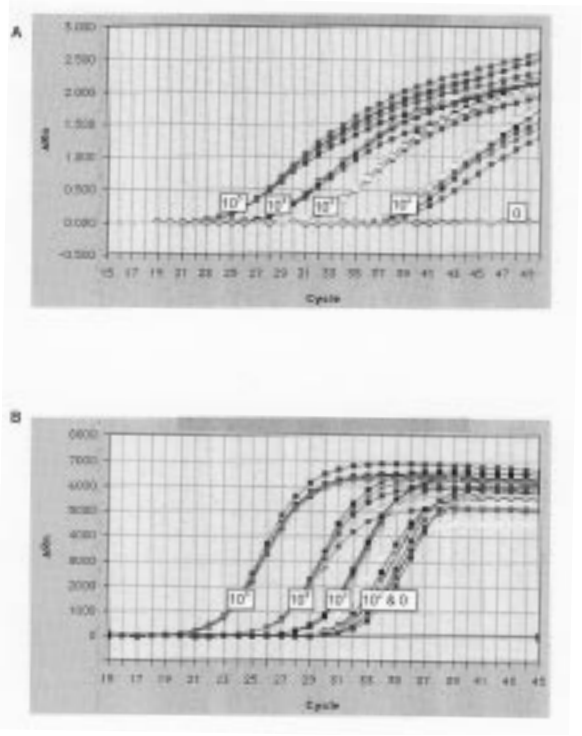

Figure 2. ABI 7700 linear amplification plots for 6 replicates of 5 plasmid copy numbers (1 = 10^5, 2 = 10^4, 3 = 10^3, 4 = 10^2, 5 = 0 copies). (A) TaqMan probe; (B) SYBR Green I.

0.1, 0.5, 2, and 5% (w/w) Roundup Ready soya flour (Fluka, Stevenage, UK). To determine the accuracy of the technologies, we analyzed processed food samples with known amounts of GM soya. Two baked biscuit samples supplied by RHM Technologies (Buckinghamshire, UK) containing 0.5 and 1% (w/w) Roundup Ready soya were used.

DNA was extracted from Roundup Ready soya flour reference standards and food samples. A 350 mg portion of sample was incubated at 56°C overnight with 3 mL extraction buffer [150mM NaCl, 2mM EDTA, 1% (w/v) sodium dodecyl sulfate (SDS), 10mM Tris, pH 8.0], 350 μL 5M guanidine thiocyanate, and 140 μL proteinase K (10 mg/mL). The DNA was purified with the Wizard™ DNA Cleanup kit (Promega, Madison, WI). The DNA was eluted from the resin columns in 100 μL TE buffer (10mM Tris, pH 8.0, + 0.1mM EDTA) and stored at 4°C. Standards were quantitated with a spectrophotometer and diluted to a final concentration of 10 ng/μL.

Plasmid Preparation

To study the detection of defined copy numbers of a specific endogenous plant gene, a region of the lectin gene was cloned into the pGEM plasmid (Promega). The *Le1* lectin gene (6) was PCR-amplified from 2% (w/w) Roundup Ready soya standard (Fluka) DNA. The lectin forward and reverse primers used for amplification are shown in Table 1. The 50 μL amplification reactions were performed with 2.5 U AmpliTaq Gold™ DNA polymerase (Applied Biosystems), 1X AmpliTaq Gold PCR buffer, 200 μM dNTPs, 100nM of each primer, and 50 ng DNA template. PCR reactions were run in an ABI 2400 thermocycler under the following conditions: 10 min at 96°C; and 40 cycles, 30 s at 94°C; 30 s at 54°C; and 25 s at 72°C with a final elongation step at 72°C for 3 min. The amplicon produced was cloned into the pGEM vector with the pGEM Easy kit (Promega). After transformation, clones containing the required lectin gene were identified by PCR amplification with M13For and M13Rev primers. A product of 429 bp was obtained if the lectin insert was present, compared to 222 bp if no insert was present. A 100 μL reaction was performed with 2.5 U Taq DNA polymerase (Amersham Biosciences, Buckinghamshire, UK), 1X Pharmacia PCR buffer, 50 μM dNTPs, 200nM of each primer. A bacterial colony was added with a sterile pipet tip. Reactions were then placed in an ABI 2400 thermocycler with the following PCR profile: 95°C for 5 min and 25 cycles of 95°C for 1 min, 50°C for 2 min, and 72°C for 1 min. These cycles were followed by a final elongation step of 72°C for 10 min.

DNA was extracted from PCR-positive clones containing the correct insert with a QIAprep Spin Miniprep kit (Qiagen, West Sussex, UK). The identity of the inserted DNA was veri-

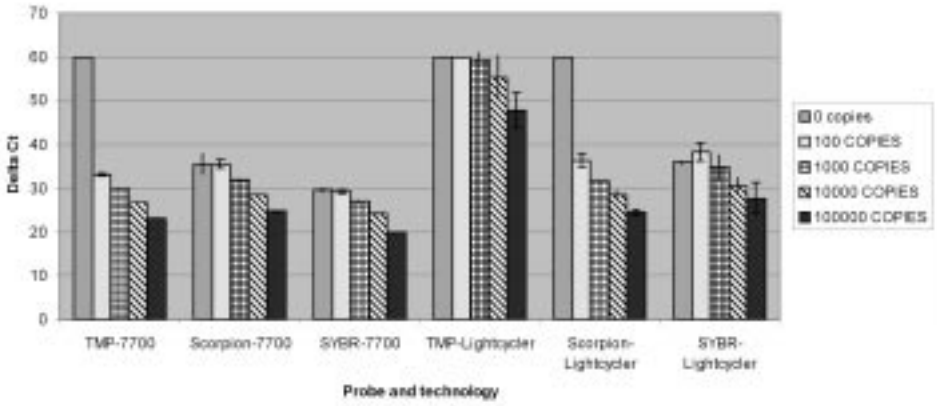

Figure 3. Plot of average Ct value ± standard deviation for the ABI 7700 and Lightcycler using the different probe detection systems.

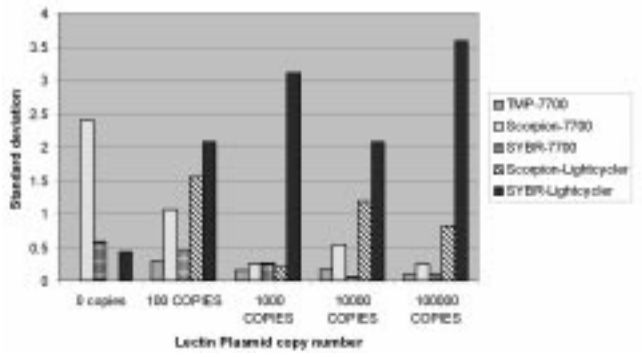

Figure 4. Plot showing the standard deviations of 6 repeat Ct values obtained for the ABI 7700 and Lightcycler platforms using the different probe detection systems tested with 5 different lectin plasmid copy numbers.

fied by sequencing the forward and reverse strands using M13For and M13Rev primers and the ABI Prism cycle sequencing kit (Applied Biosystems, Warrington, UK) on the ABI 377 fluorescent sequencer. The plasmid DNA was linearized with the restriction enzyme *Pst*I (Amersham Biosciences) by incubating 5 μg plasmid DNA with 15 U enzyme in 1X One-Phor-All buffer at 37°C overnight. The enzyme was heat-inactivated at 60°C for 15 min. The digested plasmid was dephosphorylated by addition of 0.1 U alkaline phosphatase (incubated at 37°C for 30 min and heat-inactivated at 85°C for 15 min) to ensure that the plasmid could not religate. The linearized plasmid was run on a 1.5% (w/v) agarose gel and then purified with a QIAquick gel extraction kit (Qiagen). The plasmid preparation was then quantitated by measuring the A_{260}, and the number of copies was determined (vector, 3136 bp). The plasmid was diluted to give a concentration of 2×10^4 copies/μL, which was then diluted in a 10-fold dilution series. A 5 μL of each of the plasmid dilutions was used in each PCR reaction to give final copy numbers of 10^5, 10^4, 10^3, and 10^2.

SYBR Green I

A SYBR Green I kit was used as instructed by the manufacturer (Roche Diagnostics). Reactions were performed in a volume of 20 μL containing 250nM primers, Lectin-for and Lectin-rev (Table 1), 2mM $MgCl_2$, 176 ng Taqstart antibody (Clontech, Palo Alto, CA), and 1X Roche Master Mix.

Scorpion Primers

Scorpion primers and unlabeled primer pairs were designed to amplify a portion of the *Le1* lectin gene (6; endogenous PCR system) and a region of the 35S-promoter originating from Cauliflower mosaic virus (7; GM-specific PCR) purchased from Oswel (Southampton, UK). Both Scorpion primers were 5′ end-labeled with 6-carboxyfluorescein (FAM), and each contained an internal fluorescent quencher dye, methyl red (MR), and an internal PCR stopper, hexaethylene glycol (HG).

Reactions were performed with the Lightcycler Hybridization Probes kit (Roche Diagnostics) in accordance with manufacturer's instructions in a reaction volume of 20 μL. Primers Lectin-Scor and Lectin-Rev (250mM; Table 1) were used with 2mM $MgCl_2$, 176 ng Taqstart antibody (Clontech), and 1X Roche Master Mix for endogenous PCR; 250nM of primers 35s-Scor and 35s-Rev were used with 2.5mM MgCl, 176 ng Taqstart antibody (Clontech), and 1X Roche Master Mix for GMO PCR detection.

Scorpion primers and SYBR Green I PCRs were run on the ABI Prism 7700 sequence detection system with the following program: 10 min at 95°C followed by 60 cycles, 30 s at 95°C, 30 s at 62°C, and 25 s at 72°C. These reactions were also used on the Lightcycler with the following program: 65°C, 0 s; 85°C, 0 s; a denaturation step of 95°C, 120 s, 60 cycles, comprising 95°C, 0 s; 57°C, 0 s; 72°C, 10 s (and a melt curve analysis of 50°C, 2 s, and a ramp to 95°C at 0.1°C/s).

TaqMan Probes

Primers and TaqMan probes were used as previously described (8). The endogenous PCR system detected the *Le1* lectin gene. The probe was labeled with the fluorescent reporter dye tetrachloro-6-carboxyfluorescein (TET) when used on the ABI 7700, and with FAM when used on the Lightcycler. The GM-specific PCR system detected the CP4 EPSPS gene (U.S. patent 5627061-A9). This probe was labeled on the 5′ end with FAM. The 3′ end of all probes was labeled with the fluorescent quencher dye, 6-carboxytetramethylrhodamine (TAMRA).

TaqMan reactions were performed using TaqMan PCR core reagents (Applied Biosystems). A reaction volume of 50 μL was used for reactions on the ABI 7700, and a reaction volume of 20 μL was used for reactions on the Lightcycler. All reactions on the ABI 7700 contained primers and probes for lectin and EPSPS detection; reactions on the Lightcycler contained only one set of primers and probes. Reactions contained 25nM endogenous primers (Sltm1 and Sltm2), 100nM transgenic primers (Sttmf3a and Sttmr2a), and 200nM endogenous and transgenic probes (Sltmp and Sttmpa). dATP, dGTP, and dCTP were each used at concentrations of 400 and 800 μM dUTP, 2.5 U AmpliTaq Gold DNA polymerase, 0.5 U AmpErase uracil N-glycosylase (UNG), 6mM $MgCl_2$, and 1X TaqMan buffer A.

TaqMan probe reactions were run on the ABI 7700 and the Lightcycler with the following program: 2 min at 50°C, 10 min at 95°C and 60 cycles, 15 s at 95°C, and 1 min at 60°C.

Precision and Sensitivity Analysis

To study the repeatability of the Roche Lightcycler and ABI 7700 sequence detection technologies with the TaqMan, Scorpion primers, and SYBR Green I, 5 levels of lectin plasmid, 10^5, 10^4, 10^3, 10^2, and 0 copies per reaction, were amplified in 6 replicates (Figures 1 and 2). The average cycle number at which a baseline fluorescence signal was exceeded, known as the crossing point (Ct value), was compared for each plasmid level with each method to determine the sensitivity of detection. The standard deviations for each plasmid

Table 2. Ct values for 6 replicates with 0, 10^2, 10^3, 10^4, and 10^5 copies of lectin plasmid

	One plate																		
	Run 1						Run 2						Run 3						
TMP-7700	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	*p*-value[b]
0 Copies	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	
100 Copies	34.89	35.53	35.72	35.07	35.08	35.24	34.84	34.89	35.1	34.73	35.07	35.64	35.36	35	35.02	35.23	34.47	35.45	0.529
1000 Copies	31.48	31.69	31.92	31.63	31.38	39.98	31.59	31.37	31.73	31.36	31.42	31.51	31.45	31.29	31.44	31.41	31.43	31.51	0.317
10000 Copies	28.38	28.37	28.62	28.41	28.47	28.58	28.21	28.35	28.35	28.44	28.29	28.43	28.39	28.41	28.44	28.38	28.45	28.38	0.082
100000 Copies	24.37	24.15	24.26	24.23	24.25	24.35	24.34	24.3	24.25	24.17	24.42	24.4	24.24	24.3	23.94	24.1	23.9	24.3	0.058
	Separate plates																		
TMP-7700	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	
0 Copies	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	60	
100 Copies	35.57	36.79	36.16	35.46	37.71	35.82	32.88	33.36	33.44	33.49	32.83	33.14	34.89	35.53	35.72	35.12	35.07	35.08	3E-07
1000 Copies	30.22	30.13	30.06	30.19	30.25	29.85	30.11	29.91	30.17	30.2	29.81	30.1	31.48	31.69	31.92	31.63	31.38	39.98	0.031
10000 Copies	26.55	26.64	26.19	26.71	26.51	26.51	26.63	26.45	26.77	26.95	26.63	26.64	28.38	28.37	28.62	28.41	28.47	28.58	9E-13
100000 Copies	22.87	23.07	23.12	23.06	23.08	23.11	23.1	23.24	22.99	23.12	23.08	23	24.37	24.15	24.26	24.23	24.25	24.35	2E-13
	Separate runs																		
Scorpion primers-Lightcycler	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	Ct-1	Ct-2	Ct-3	Ct-4	Ct-5	Ct-6	
0 Copies	60	60	44.56	60	60	60	60	59.39	28.48	60	60	60	60	59.79	60	60	60	60	0.549
100 Copies	36.6	38.71	35.21	35.05	35.1	37.69	60	60	60	41.01	60	60	39.32	39.75	39.35	36.94	37.18	37.86	2E-06
1000 Copies	31.37	31.42	31.9	31.43	31.62	31.66	37.35	35.96	32.93	34.87	35.09	34.83	34.65	34.61	33.04	33.94	33.99	33.54	2E-05
10000 Copies	29.78	27.4	27.6	30.16	27.77	28.24	34.5	32.3	31.83	29.48	32.19	32.29	31.9	31.51	30.13	31.03	30.2	30.66	4E-04
100000 Copies	25.83	24.7	23.87	23.9	23.6	24.21	29.77	26.76	26.42	26.23	28.7	29.03	27.96	27.5	25.78	27.16	27.13	26.81	1E-04

[a] Tests were repeated 3 times on the same plate in the 7700 and in 3 separate runs for the ABI 7700 and the Lightcycler.

[b] The *p*-value is calculated using a single-factor ANOVA test to determine if there is a significant difference between the 6 replicates from the 3 runs.

Table 3. Percentage GMO soya relative to total soya for 2 baked biscuit samples[a]

Baked sample	Actual % GMO soya relative to total soya	Calculated % GMO relative to total soya	
		TaqMan probes	Scorpion primers
1	1.0	1.4 ± 0.50	0.6 ± 0.38
2	0.1	0.3 ± 0.32	0.2 ± 0.22

[a] The actual results are compared with those obtained with TaqMan probes on the ABI 7700 and Scorpion primers on the Roche Lightcycler ± 1 SD.

level with each method were compared to determine if there was a significant difference in the variability of Ct values between methods (Figures 3 and 4).

Inter- and Intra-Run Variability Analysis

Using the most precise probe system with the ABI 7700 and Roche Lightcycler, we studied the variability between repeat runs. Five plasmid copy numbers were studied. For the precision testing (10^5, 10^4, 10^3, 10^2, and 0 copies), these copy numbers were repeated 6 times in each batch. For the ABI 7700, these 6 repeats of the 5 plasmid levels were repeated 3 times on the same amplification run and 3 times on separate runs. For the Roche Lightcycler, 3 repeat runs were performed for 6 repeats of the 5 plasmid concentrations (Table 2).

Determination of GM Soya Content of Food Samples

Using TaqMan probes for the ABI 7700, the lectin gene and the EPSPS gene were amplified in a dual assay using 2 conventional sets of PCR primers and 2 fluorescent probes in the same tube. Using Scorpion primers on the Roche Lightcycler, the lectin gene and the 35S-promoter were amplified in 2 separate assays. For each system, the standards and samples were amplified during the same run. The standards were used to produce a calibration curve, plotting Delta Ct ($Ct_{GMO} - Ct_{Lectin}$) vs the logarithm of the concentration. The equation of this standard curve was used to determine the percentage of GM soya relative to total soya for 2 baked biscuit samples with known GMO content (supplied by RHM Technologies).

Statistical Analysis

In the initial precision study of all combinations of detection technologies, standard deviations (SDs) were calculated and compared using a 2-tailed *F*-test. To determine if variation between 3 separate batches of 6 replicate Ct values was significant, a single factor analysis of variance (ANOVA) test was used. Results were considered significant at the 95% confidence level.

Results and Discussion

Precision and Sensitivity of SYBR Green I, Scorpion Primers, and TaqMan Probes with the Lightcycler and ABI 7700 Platforms

Amplification plots showing the 6 replicates for each copy number using the Scorpion primer on the Lightcycler and TaqMan probe and SYBR Green I on the ABI 7700 are shown in Figures 1 and 2. The average Ct values with SDs plotted as error bars are shown in Figure 3. The SDs for each plasmid number for each method are shown in Figure 4.

The cycle number at which the baseline fluorescence signal was exceeded (Ct value) was lower when SYBR Green I dye was used with ABI 7700, suggesting that this method of detection is more sensitive than either the TaqMan or Scorpion systems. However, SYBR Green I dye is not sequence-specific, and for the ABI 7700 the Ct values obtained for 10^2 copies and 0 copies was not significantly different at the 95% confidence level. For the Lightcycler there was no significant difference between the Ct values obtained for 10^3 copies and 0 copies, probably because of the detection of primer-dimer in the reaction. Detection of 10^2 and 10^3 copies is therefore not possible using this detection method on the ABI 7700 and Lightcycler, respectively.

SYBR Green I detection on the Roche Lightcycler showed the greatest degree of variability in Ct value for each of the plasmid levels tested (Figure 4). This variability was significantly different at the 95% confidence level from the variabil-

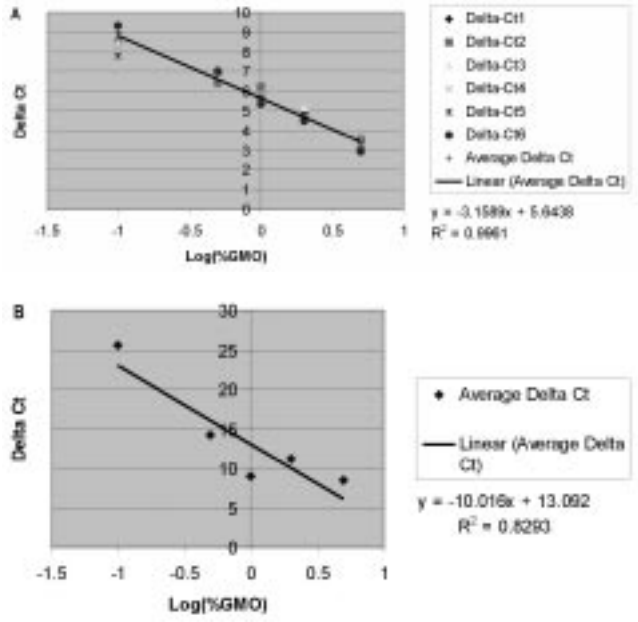

Figure 5. Standard curves plotting Delta Ct values versus log (% GMO) for Fluka soya standards using soya endogenous and GMO-specific PCR detection. The equation of the line for average Delta Ct is shown. (A) Standard curve obtained from PCR amplification using TaqMan probes on the ABI 7700; (B) standard curve obtained using Scorpion primers on the Roche Lightcycler.

ity obtained with TaqMan probes, Scorpion primers, and SYBR Green I on the ABI 7700, and from Scorpion primers used on the Roche Lightcycler for most plasmid levels tested. The smallest variation in Ct value was obtained with either TaqMan probes or SYBR Green I on the ABI 7700, with the variation in Ct value between the 2 not significantly different at the 95% confidence level. Use of the Scorpion primer on the ABI 7700 showed significant differences in variability for a number of the plasmid levels tested compared with TaqMan and SYBR Green I. Differences in variability were significant at the 0, 10^4, and 10^5 copy levels compared with SYBR Green I on the ABI 7700, and at the 10^2, 10^4, and 10^5 copy levels compared with the TaqMan probe on the ABI 7700. No significant differences in variability were seen for the Scorpion primers on either the ABI 7700 or the Roche Lightcycler except at the 10^5 copy number level.

Inter- and Intra-Run Variability

Table 2 shows the Ct values for each of the 5 plasmid levels in replicates of 6 for each of the 3 batches. Variations between the 3 runs compared by single factor ANOVA tests for each of the plasmid levels are shown as *p*-values. Inter-run variation was significant between batches run separately on the ABI 7700 and on the Roche Lightcycler. This was in contrast to the 3 batches repeated in the same run on the ABI 7700, which showed no significant variation between batches for each of the 5 plasmid levels tested.

Determination of GMO Content for Unknown Baked Samples

A summary of the results obtained for determination of percentage GMO content using TaqMan probes on the ABI 7700 and Scorpion primers on the Roche Lightcycler is shown in Table 3. Both methods gave a degree of inaccuracy in determining the % GMO content of the unknown samples. The difference in result and SDs obtained with the different methods did not show one method to be clearly superior to the other, although the standard curve obtained with the TaqMan probes used on the ABI 7700 showed a higher degree of linearity (Figure 5).

Conclusions

Although SYBR Green I was more sensitive than the sequence-specific detection systems, it was unable to discriminate between 0 copies and 10^2/10^3 copies due to the detection of primer-dimer. Therefore, the advantage of increased sensitivity with this detection method is outweighed by the lack of specificity.

Investigation of the degree of variability of Ct values for 5 plasmid copy numbers concentrations revealed that TaqMan probes and SYBR Green I used on the ABI 7700 resulted in the least degree of variability producing the most precise Ct values. With the Roche Lightcycler, Scorpion primers showed the least degree of variability for Ct values, but the variability obtained was still significantly greater than that for TaqMan and SYBR Green I used on the ABI 7700.

Intra-run variability on the ABI 7700 showed no significant variation between batches compared with significant ($p < 0.05$) variation between separate runs on both the ABI 7700 and the Roche Lightcycler. Therefore, standards and samples should always be run in the same amplification. With the Roche Lightcycler, only 32 PCRs can be performed in each run compared with 96 for the ABI 7700. To perform 6 replicate reactions, only one sample can be run at a time, which is extremely limiting and restricts the use of this method on the Lightcycler. Nonetheless, an amplification run is very rapid on the Lightcycler, taking <1 h compared with 3 h for the ABI 7700.

The TaqMan probes used on the ABI 7700, and Scorpion primers used on the Lightcycler showed little difference in accuracy for determination of percentage GMO content for 2 baked samples. However, in selecting the method of choice for quantitative GMO detection, the greater precision, more accurate standard curve, and greater number of repeats possible must be taken into consideration. Therefore, the use of TaqMan probes on the ABI 7700 offers significant advantages.

Acknowledgments

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