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Event 5307 Maize:

Validation of Real-time, Event-specific Polymerase
Chain Reaction Method

Data Requirements: Not applicable

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Study Completion Date: October 22, 2010

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No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 10 (d) (1) (A), (B), or (C).

Company: *Syngenta Seeds, Inc.*

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The provisions of Good Laboratory Practices Standards (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act do not apply to this report because it summarizes the validation of an analytical method. These analyses were performed according to accepted scientific practices, and relevant study records (including raw data) have been retained.

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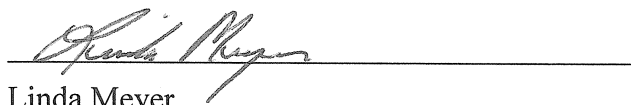
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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

5′	five prime
adh1	alcohol dehydrogenase 1 gene
bias	relative deviation from the expected value
BLASTN	Basic Local Alignment Search Tool for Nucleotides
bp	base pair
CT	cycle threshold
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
ecry3.1Ab	eCry3.1Ab gene
eCry3.1Ab	eCry3.1Ab protein
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
LOD	limit of detection
LOQ	limit of quantification
manA	phosphomannose isomerase gene
mCry3A	modified Cry3A protein
n	number
NCBI	National Center for Biotechnology Information
ng	nanogram
PCR	polymerase chain reaction
pmi	phosphomannose isomerase gene
PMI	phosphomannose isomerase enzyme
RSD_r	relative repeatability standard deviation
SD	standard deviation
US EPA	United States Environmental Protection Agency
™	trademark
®	registered
Δ CT	delta CT (difference in CT values)
μl	microliter

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SUMMARY

Using the techniques of modern molecular biology, Syngenta has transformed maize (*Zea mays)* to produce Event 5307 maize, a new cultivar that has insecticidal activity against certain corn rootworm (*Diabrotica*) species.

A real-time, event-specific polymerase chain reaction (PCR) method was developed to detect and quantify Event 5307 deoxyribonucleic acid (DNA) extracted from grain and seed samples. This method is described in a 2010 report authored by H. Hart, titled *"Event 5307 Maize: Real-time, Event-specific Polymerase Chain Reaction Method"* (Syngenta Report No. SSB-237-10). The method consists of a maize-specific PCR method as a reference, and an event-specific PCR method for detection of Event 5307 maize. This method can be used to determine the relative content of Event 5307 maize DNA in proportion to total maize DNA in samples.

The real-time, Event 5307-specific PCR method demonstrated specificity for DNA containing the Event 5307 insert. The method performed in a linear manner with an acceptable level of accuracy and precision over the entire dynamic range of 0.08% to 5.0% Event 5307 DNA. This method can detect Event 5307 DNA in samples containing at least 0.04% Event 5307 DNA and can quantitate Event 5307 DNA in samples containing at least 0.08% Event 5307 DNA. The PCR method performed acceptably when transferred to additional laboratories. All performance characteristics evaluated met the defined acceptance criteria.

Based on these results, the real-time, Event 5307-specific PCR method is suitable for detection and quantitation of Event 5307 DNA extracted from grain and seed.

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INTRODUCTION

Using the techniques of modern molecular biology, Syngenta has transformed maize (*Zea mays*) to produce Event 5307 maize, a new cultivar that has insecticidal activity against certain corn rootworm (*Diabrotica*) species. Maize plants derived from transformation Event 5307 ("5307 maize") contain the gene *ecry3.1Ab* encoding an eCry3.1Ab protein and the gene *pmi* (also known as *manA*) encoding the enzyme phosphomannose isomerase (PMI). The eCry3.1Ab protein is an engineered chimera of modified Cry3A (mCry3A) and Cry1Ab proteins. The gene *pmi* was obtained from *Escherichia coli* strain K-12 and the protein it encodes was utilized as a plant selectable marker during development of 5307 maize.

A real-time, event-specific polymerase chain reaction (PCR) method was developed to detect and quantify 5307 deoxyribonucleic acid (DNA) extracted from grain and seed samples (Hart 2010). The method consists of a maize-specific PCR method as a reference, and an event-specific PCR method for detection of 5307 maize DNA. This method determines the relative content of 5307 maize DNA in proportion to total maize DNA in samples. The method was used in conjunction with a DNA extraction method which yields DNA of sufficient purity and quantity.

Validation acceptance criteria were set prior to experimental verification for the following parameters: specificity, precision, accuracy, dynamic range, limit of detection (LOD), limit of quantification (LOQ), amplification efficiency, R^2 coefficient, robustness, and inter-laboratory transferability.

MATERIALS AND METHODS

Plant Material

Hybrid maize seed hemizygous for the 5307 trait were used in this study.

Table 1. Plant material

Seed identification	Material identification	Pedigree code
5307 maize	07MG005417	NP2171xNP2460/5307
Nontransgenic maize	07MG002998	NP2171xNP2460

Calibration Samples

The calibration samples (Standard 1 to Standard 5) contained 10%, 5%, 1%, 0.5%, and 0.1% of 5307 DNA mixed with nontransgenic maize genomic DNA. The DNA concentration in each calibration sample was 50 ng/µl with a total DNA content of 250 ng/reaction. The dilution scheme of the calibration samples and the corresponding total genomic DNA content in the calibration samples are shown in Table 2.

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Table 2. Dilution scheme of the calibration samples

Samples	Standard 1	Standard 2	Standard 3	Standard 4	Standard 5
Total DNA content	250 ng	250 ng	250 ng	250 ng	250 ng
5307 DNA content	25 ng	12.5 ng	2.5 ng	1.25 ng	0.25 ng
Nontransgenic DNA content	225 ng	237.5 ng	247.5 ng	248.75 ng	249.75 ng

Reference Samples

The reference samples contained 5.0%, 2.0%, 0.90%, 0.50%, 0.08% and 0.040% of 5307 DNA mixed with nontransgenic maize genomic DNA. The DNA concentration in each reference sample was 50 ng/µl with a total DNA content of 250 ng/reaction. The dilution scheme of the reference samples and the corresponding total genomic DNA content in the reference samples are shown in Table 3.

Table 3. Dilution scheme of the reference samples

Samples	5.0%	2.0%	0.90%	0.50%	0.08%	0.040%
Total DNA content	250 ng	250 ng	250 ng	250 ng	250 ng	250 ng
5307 DNA content	12.5 ng	5 ng	2.25 ng	1.25 ng	0.2 ng	0.1 ng
Nontransgenic DNA content	237.5 ng	245 ng	247.75 ng	248.75 ng	249.8 ng	249.9 ng

Statistical Analysis

Mean, standard deviation (SD), slope, intercept, R^2 coefficient, and amplification efficiency calculations were performed using Microsoft Office Excel® 2000 software.

Real-time, 5307-specific PCR Method

Quantification of 5307 DNA was performed according to the method described in Hart (2010). Unless otherwise noted, samples containing 5307 DNA were diluted in nontransgenic maize DNA and a total DNA content of 250 ng/reaction was used.

Real-time, Maize-specific Reference PCR Method

A maize-specific PCR method (Hernandez *et al.* 2004), which amplifies a 135 base pair (bp) fragment of the alcohol dehydrogenase 1 gene (*adh1*) of maize (Entrez® Database Accession No. AY691949 [NCBI 2010]), is used as a reference method. Use of the *adh1*-specific maize reference method was performed according to the method described in Hart (2010) and Herandez *et al*. (2004).

General Considerations for Performing PCR Analysis

In order to ensure the correct performance of the real-time, 5307-specific PCR method and to avoid any risk of contamination, suitable laboratory facilities with dedicated work spaces for different steps were used. All handling of PCR reagents and controls was performed using dedicated equipment to minimize the chance of cross-contamination. All materials used (*e.g*., vials, containers, pipette tips, *etc*.) were suitable for PCR and

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molecular biology applications. They were deoxyribonuclease (DNase)-free, DNA-free, sterile, and unable to absorb protein or DNA. Handling of all reagents and controls was conducted in a manner to preclude contamination of reagents or controls with exogenous DNA or DNases. The PCR reagents were stored and handled in a separate room from the genomic DNA samples and protected in equipment where no nucleic acids (with the exception of PCR primers or probes) or DNA degrading or modifying enzymes had been handled previously.

Cost and Time Required to Perform the Real-time, 5307-specific PCR Method

Twelve samples can be analyzed in triplicate per 96-well PCR plate. Assuming consumable costs (*e.g.,* plasticware, reagents) of about 150 Euros per plate, the cost per sample result was approximately 13 Euros (2009 estimated prices). The analysis process, which included set-up of PCR reactions, automated PCR amplification, and real-time measurement of fluorescence signals on the ABI PRISM® 7900HT Sequence Detection System, in addition to the subsequent computer assisted data evaluation, required a total of four hours.

STUDY DESIGN

Specificity of the Real-time, 5307-specific PCR method

Basic Local Alignment Search Tool for Nucleotides (BLASTN) analysis

The 5307-specific amplicon sequence was compared to the National Center for Biotechnology Information (NCBI) Entrez® Nucleotide Database (NCBI 2009) to identify any similarity with known sequences.

> **Acceptance criterion:** The absence of a 100% match of the entire amplicon with other sequences.

PCR analysis

All specificity tests were performed in triplicate (n = 3). In the specificity PCR analysis, 100 ng of genomic DNA was used in each sample. Reactions with cycle threshold (CT) values lower than 40 and with an amplification curve were defined as positive, whereas reactions with CT values of 40 and no amplification curve were defined as negative. Cycle threshold is defined as the cycle number at which the fluorescence generated within a reaction crosses a threshold at which a reaction reaches a fluorescence intensity above background. Amplification curve is defined as the measure of the fluorescence generated during amplification.

> **Acceptance criterion:** The presence of amplification in the samples that did contain 5307 DNA and an absence of amplification in the samples that did not contain 5307 DNA.

Precision/Accuracy/Dynamic Range/LOQ/LOD

In order to determine precision, accuracy, dynamic range, LOQ, and LOD the following experimental design was carried out in eight independent tests.

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A calibration curve was produced by plotting mean Δ CT values (*i.e.,* difference in sample and control CT values) of calibration samples against the logarithm of the respective percent 5307 DNA contents; the slope (a) and the intercept (b) of the calibration curve (y = ax + b) were then used to calculate the mean percent 5307 DNA content of the reference samples based on their normalized Δ CT values.

Duplicate negative control samples containing no template DNA verified the purity of the reagents for both the 5307-specific and *adh1*-specific PCR methods in each test. Each calibration sample was analyzed in duplicate in each test.

Data analysis used an appropriate individual baseline setting for the *adh1*-specific PCR method and for the 5307-specific PCR method. The threshold values were: 0.2 (*adh1*-specific PCR method) and 0.3 (5307-specific PCR method) on the ABI PRISM® 7900 HT Sequence Detection System.

Triplicates of the 5.0%, 2.0%, 0.9%, 0.5%, and 0.08% 5307 DNA samples were included in each test. The quantification results were determined by calculating the mean of two test results (each test included triplicates of each DNA sample) for the 5.0%, 2.0%, 0.9%, 0.5%, and 0.08% 5307 DNA samples. The eight independent tests resulted in 16 results for the 5.0%, 2.0%, 0.9%, 0.5%, and 0.08% 5307 DNA samples, and eight test results for the 0.040% 5307 DNA sample.

For each of the five (5.0%, 2.0%, 0.9%, 0.5%, and 0.08%) reference samples, the mean, the relative deviation from the expected value as well as the standard deviation (stdev), and the relative repeatability standard deviation (RSD_r) of the quantification results were calculated to determine accuracy and repeatability.

Precision (Relative repeatability standard deviation, RSD_r)

Precision is defined as the standard deviation of test results obtained under repeatability conditions. Repeatability conditions are conditions where test results are obtained with the same method, on identical test items, in the same laboratory, by the same operator, using the same equipment within short intervals of time (*i.e,.* intra-laboratory repeatability.

> **Acceptance criterion:** $RSD_r \leq 25\%$ over the whole dynamic range (0.08% to 5.0% 5307 DNA).

Accuracy (Bias)

Accuracy is defined as the closeness of agreement between the average value obtained from a large series of test results and the accepted reference value.

> **Acceptance criterion:** Accuracy within +/- 25% of the accepted reference value over the whole dynamic range (0.08% to 5.0% 5307 DNA).

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Dynamic range

Dynamic range is defined as the range of concentrations of 5307 DNA over which the method performs in a linear manner with an acceptable level of accuracy and precision.

> **Acceptance criterion:** The dynamic range covers 0.08% to 5.0% 5307 DNA concentrations.

LOQ

LOQ is defined as the lowest amount or concentration of 5307 DNA in a sample that can be reliably quantified with an acceptable level of precision and accuracy.

> **Acceptance criterion:** $RSD_r \leq 25\%$ and quantification results within +/- 25% of the accepted reference value. The LOQ of the 5307-specific PCR method should be at least 0.08% 5307 DNA.

LOD

LOD is defined as the lowest amount or concentration of 5307 DNA in a sample which can be detected reliably, but not necessarily quantified.

> **Acceptance criterion:** Detection of 5307 DNA in at least 95% of the 5307 DNA samples. The LOD of the 5307-specific PCR method should be at least 0.040% 5307 DNA.

Amplification Efficiency and R^2 Coefficient

Linear regression analysis of the real-time, 5307-specific PCR method CT values versus log[% 5307 DNA] was performed to assess amplification efficiency (*i.e.,* the rate of amplification that leads to a theoretical slope of -3.32 with an efficiency of 100% in each cycle) and R^2 coefficient (*i.e.,* correlation coefficient of a standard curve obtained by linear regression analysis) of the 5307-specific PCR method. The regression lines of the standards of eight independent runs were evaluated and the regression parameters, including slope, intercept, and R^2 coefficient, were determined. The efficiency of the amplification was calculated by the following equation: Efficiency = $[10^{(-1/\text{slope})}]$ -1.

Linear regression analysis of Δ CT values versus log[% 5307 DNA content] was performed to assess Efficiency and R^2 coefficient of the Δ CT based 5307 PCR method. The regression lines of the standards of eight independent tests were evaluated and the regression parameters, including slope, intercept, and R^2 were determined. The efficiency of the amplification was calculated by the following equation: Efficiency = $[10^{(-1/\text{slope})}]$ -1.

> **Acceptance criterion (Efficiency):** The average value of the slope is in the range of -3.1 $\geq$ slope $\geq$ -3.6.

> **Acceptance criterion (R^2):** The average value of R^2 is $\geq$ 0.98.

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Robustness

Variation of concentration of the master mix

The concentration of the master mix was varied +20% and -20% of the standard concentration to assess the performance of both the 5307-specific and *adh1*-specific PCR methods with changes in the concentration of major reaction components. Samples containing 0.08%, 0.90%, and 5.0% 5307 DNA were analyzed on the ABI PRISM® 7900 HT Sequence Detection System.

> **Acceptance criterion:** Results are within +/- 30% of the accepted reference value.

Variation of annealing temperature

The annealing temperature of the 5307-specific PCR method was varied to 58°C and 62°C. Samples containing 0.08%, 0.90%, and 5.0% 5307 DNA were analyzed on the ABI PRISM® 7900 HT Sequence Detection System.

> **Acceptance criterion:** Results are within +/-30% of the accepted reference value.

Performance on different instrument types

Samples containing 0.08%, 0.90%, and 5.0% 5307 DNA were analyzed on the ABI PRISM® 7700, Applied Biosystems™ 7500 Fast Real-Time PCR system and Stratagene Mx3005P™ QPCR detection system to assess the influence of different real-time PCR instruments. Data analysis for the ABI 7500 Fast Real-Time PCR system used a baseline setting ranging from cycle three to the cycle number where the first amplification curve crosses the threshold minus three (CT – 3). Data analysis for the ABI PRISM® 7700 Sequence Detection System were carried out with a baseline setting from cycle 3 to 15. Data analysis for the Stratagene Mx3005P™ QPCR detection system was carried out with an adaptive baseline setting.

> **Acceptance criterion:** Results are within +/- 30% of the accepted reference value.

Inter-laboratory Transferability

In order to assess the performance of the real-time, 5307-specific PCR method under reproducibility conditions (inter-laboratory), two quantification tests were performed at the following laboratories:

Syngenta Biotechnology, Inc. (Laboratory 1)
3054 East Cornwallis Road
PO Box 12257
Research Triangle Park, NC 27709-2257 USA

Eurofins GeneScan USA, Inc. (Laboratory 2)
2315 North Causeway Blvd.
Metairie, LA 70001 USA

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Samples containing 0.08% to 5.0% 5307 DNA were analyzed in triplicate on different sequence detection systems.

Acceptance criteria: Results are within +/- 35% of the accepted reference value at 5.0% and 0.90% 5307 DNA; results are within +/- 50% of the accepted reference value at 0.08% 5307 DNA.

RESULTS

Specificity of the 5307-specific PCR Method

BLASTN analysis

Figure 1 contains the results from the BLASTN analysis with the 5307-specific amplicon sequence. Matches of the 5' end of the amplicon with plasmid sequences were identified. This is expected as the 5' end of the 5307-specific amplicon contains 5307 insert sequence. No significant match of the entire amplicon was found; therefore, the specificity of the method as determined by BLASTN analysis is acceptable.

Figure 1. Results of BLASTN analysis with the amplicon sequence of the 5307-specific PCR method
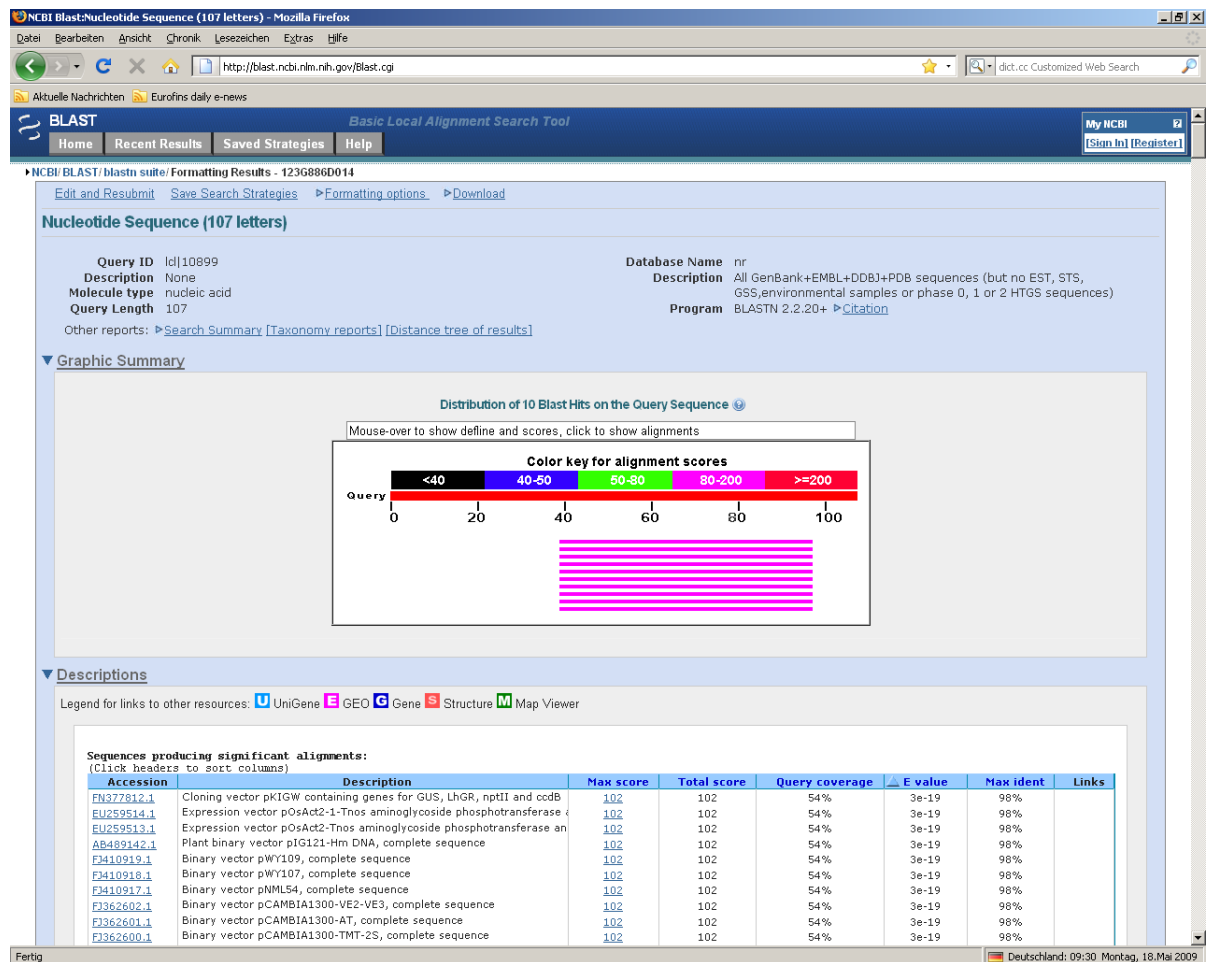
PCR analysis

Results of the PCR analysis demonstrated that there was amplification in the samples that contained 5307 DNA and no amplification in the samples that did not contain 5307 DNA (Table 4). Therefore, the specificity of the 5307-specific PCR method as determined by PCR analysis is acceptable.

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Table 4. Specificity results of PCR analysis with the 5307-specific PCR method

Template DNA sample source	Result
5307 maize	Positive
Bt11 maize	Negative
NK603 maize	Negative
MON88017 maize	Negative
MON863 maize	Negative
MON810 maize	Negative
MON89034 maize	Negative
3272 maize	Negative
TC1507 maize	Negative
MIR604 maize	Negative
Bt176 maize	Negative
GA21 maize	Negative
T25 maize	Negative
DAS-59122-7 maize	Negative
Nontransgenic maize	Negative
Nontransgenic soybean	Negative
Nontransgenic canola	Negative
Nontransgenic rice	Negative
Nontransgenic wheat	Negative
Nontransgenic potato	Negative
Nontransgenic sugar beet	Negative
Nontransgenic cotton	Negative

Precision/Accuracy/Dynamic Range/LOQ/LOD

Table 5 depicts the results of the precision, accuracy, dynamic range, LOQ, and LOD of the experiments.

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Table 5. Quantification results of eight independent PCR tests under repeatability conditions for 5307 maize DNA

5307 level (%)	Test 1 (%)	Test 2 (%)	Test 3 (%)	Test 4 (%)	Test 5 (%)	Test 6 (%)	Test 7 (%)	Test 8 (%)	Mean (%)	Bias (%)	SD (%)	RSD$_r$ (%)
5.0	5.8	5.9	4.6	4.5	6.0	5.0	4.8	5.1	5.1	2.0	0.55	10.8
	5.8	4.2	5.3	5.3	4.6	4.6	5.0	5.0				
2.0	2.4	2.0	1.9	2.1	2.3	2.4	2.0	2.2	2.1	5.0	0.27	12.9
	2.2	2.5	1.8	1.9	2.6	2.2	1.7	1.8				
0.90	1.01	0.89	0.92	0.84	0.81	0.73	1.04	1.01	0.91	1.1	0.107	11.8
	0.82	0.85	0.93	0.96	0.90	1.11	0.74	0.98				
0.50	0.59	0.48	0.44	0.44	0.46	0.49	0.41	0.47	0.46	-8.0	0.057	12.4
	0.45	0.48	0.46	0.54	0.51	0.45	0.38	0.35				
0.08 (LOQ)	0.077	0.080	0.054	0.056	0.078	0.050	0.045	0.053	0.067	-16.3	0.0164	24.5
	0.067	0.054	0.092	0.070	0.102	0.076	0.052	0.073				
0.040 (LOD)	0.033	0.034	0.031	0.041	0.039	0.039	0.058	0.030				

Precision (Relative repeatability standard deviation, RSD$_r$)

The relative standard deviation (RSDr) for all samples containing 0.08% to 5.0% 5307 DNA ranged from 10.8% to 24.5% under repeatability conditions. Therefore, the precision of the method is acceptable.

Accuracy (Bias)

The relative deviation of the mean from the expected value ranged from -16.3% to 5.0% for samples containing 0.08% to 5.0% 5307 DNA. Therefore, the accuracy of the method is acceptable.

Dynamic range

The real-time, 5307-specific PCR method performs in a linear manner over the range of 0.08% to 5.0% 5307 DNA with an acceptable level of accuracy and precision. Therefore, the dynamic range is acceptable.

LOQ

The mean RSD$_r$ was $\leq 25\%$ and the quantification results were within +/- 25% of the accepted reference value for the sample containing 0.08% 5307 DNA. Therefore, the LOQ was at least 0.08% and was acceptable.

LOD

The method detected 5307 DNA in all of the samples containing 0.040% 5307 DNA. Therefore, the LOD was at least 0.040% and was acceptable.

Amplification Efficiency and R^2 Coefficient

Tables 6 and 7 summarize the amplification efficiency and R^2 coefficient data. For the 5307-specific method, the average value of the slope is -3.23 and the average value of R^2 coefficient is 0.99. For the *adh1*-specific method, the average value of the slope is -3.13 and the average value of R^2 coefficient is 0.99. Therefore, the efficiency and R^2 coefficient are acceptable.

Table 6. Regression parameters and PCR efficiencies of the 5307-specific PCR method regression lines

	Slope	Intercept	R^2 Coefficient	Efficiency
Test 1	-3.23	30.0	0.99	1.04
Test 2	-3.28	29.7	1.00	1.02
Test 3	-3.14	29.9	1.00	1.08
Test 4	-3.19	30.2	1.00	1.06
Test 5	-3.22	29.9	0.98	1.05
Test 6	-3.29	30.0	0.99	1.01
Test 7	-3.42	30.3	0.99	0.96
Test 8	-3.07	30.2	0.99	1.12
Mean	-3.23	30.0	0.99	1.04

Table 7. Regression parameters and PCR efficiencies of the calibration curves based on Δ CT values of the calibration samples

	Slope	Intercept	R^2 Coefficient	Efficiency
Test 1	-3.17	7.0	0.99	1.07
Test 2	-3.21	6.8	1.00	1.05
Test 3	-3.00	6.9	1.00	1.15
Test 4	-3.03	7.0	1.00	1.14
Test 5	-3.14	6.9	0.99	1.08
Test 6	-3.14	7.0	0.99	1.08
Test 7	-3.35	7.0	0.99	0.99
Test 8	-3.01	6.8	1.00	1.15
Mean	-3.13	6.9	0.99	1.09

Robustness

Variation of concentration of the master mix

Table 8 summarizes the results from performing the real-time, 5307-specific PCR method with various concentrations of master mix. The data are presented as the mean of triplicates. The results are within +/- 30% of the accepted reference value. Therefore, the robustness of the 5307-specific PCR method as determined by varying the concentration of the master mix is acceptable.

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Table 8. Quantification results at +/- 20% of master mix concentration

	Expected value (% 5307 DNA)	Quantification results (% 5307 DNA)	Relative deviation from expected value (%)
Master mix (+ 20%)	0.08	0.086	7.5
	0.90	1.02	13.3
	5.0	4.9	-2.0
Master mix (-20%)	0.08	0.068	-15.0
	0.90	0.77	-14.4
	5.0	4.3	-14.0

Variation of annealing temperature

Table 9 summarizes the results from performing the real-time, 5307-specific PCR method with various annealing temperatures. The data are presented as the mean of triplicates. The results are within +/-30% of the accepted reference value. Therefore, the robustness of the 5307-specific PCR method as determined by varying the annealing temperature is acceptable.

Table 9. Quantification results using different annealing temperatures

	Expected value (% 5307 DNA)	Quantification results (% 5307 DNA)	Relative deviation from expected value (%)
Annealing temperature of 58°C	0.08	0.061	-23.8
	0.90	0.96	6.7
	5.0	4.9	-2.0
Annealing temperature of 62°C	0.08	0.058	-27.5
	0.90	0.82	-8.9
	5.0	5.4	8.0

Performance on different instrument types

Table 10 summarizes the results from performing the real-time, 5307-specific PCR method on different instrument types. The data are presented as the mean of triplicates. The results are within +/- 30% of the accepted reference value. Therefore, the robustness of the 5307-specific PCR method as determined by performance on different instrument types is acceptable. The quantitative PCR method for detection of 5307 genomic DNA was established, optimized, and validated on an ABI PRISM® 7900HT Sequence Detection System. However, if the ABI PRISM® 7900 HT Sequence Detection System is unavailable, the method can be applied on a different platform with further optimization and adaptation.

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Table 10. Quantification results using different instrument types

	Expected value (% 5307 DNA)	Quantification results (% 5307 DNA)	Relative deviation from expected value (%)
ABI 7500 Fast	0.08	0.057	-28.8
	0.90	0.90	0.0
	5.0	5.4	8.0
ABI PRISM® 7700	0.08	0.071	-11.3
	0.90	0.83	-7.8
	5.0	5.6	12.0
Stratagene Mx3005P™	0.08	0.083	3.8
	0.90	1.03	14.4
	5.0	4.0	-20.0

Inter-laboratory Transferability

Table 11 summarizes the reproducibility results from quantifications performed in duplicate for different percentages of 5307 DNA at two different laboratories. The results are within +/- 35% of the accepted reference value at 5.0% and 0.90% 5307 DNA. Additionally the results are within +/- 50% of the accepted reference value at 0.08% 5307 DNA. These data indicate that the inter-laboratory transferability of the real-time, 5307-specific PCR method is acceptable.

Table 11. Quantification results for reproducibility tests

	Expected value (% 5307 DNA)	Quantification results (% 5307 DNA)	Relative deviation from expected value (%)
Laboratory 1 (ABI PRISM® 7900 HT)	0.08	0.057	-28.8
	0.90	0.89	-1.1
	5.0	4.7	-6.0
Laboratory 2 (Stratagene Mx3005P™)	0.08	0.083	3.8
	0.90	1.05	16.7
	5.0	5.1	2.0

Data Quality and Integrity

No circumstances occurred during the conduct of this method validation that would have adversely affected the quality or integrity of the data generated.

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CONCLUSIONS

A quantitative real-time, event-specific PCR method was developed to detect 5307 DNA extracted from grain and seed samples.

The real-time, 5307-specific PCR method demonstrated specificity for DNA containing the 5307 insert. The method performed in a linear manner with an acceptable level of accuracy and precision over the entire dynamic range of 0.08% to 5.0% 5307 DNA content. This method can detect 5307 DNA in samples containing at least 0.04% 5307 DNA and can quantitate 5307 DNA in samples containing at least 0.08% 5307 DNA. The PCR method performed acceptably when transferred to other laboratories. All performance characteristics evaluated met the defined acceptance criteria.

Based on these results, the real-time, 5307-specific PCR method is suitable for the quantitative detection of 5307 DNA extracted from grain and seed.

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RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

CONTRIBUTING SCIENTISTS

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