SUMMARY

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for public release after registration)

STUDY TITLE

Polymerase Chain Reaction (PCR) Method Validation for the Detection of Maize Containing Event TC1507

DATA REQUIREMENTS

N/A

AUTHORS

I. Lamb

A. Nemeth

STUDY COMPLETED ON

09/06/2001

PERFORMING LABORATORY

GeneScan Analytics GmbH
GeneScan Europe AG
Engesserstrasse 4b, 79108 Freiburg
Germany

LABORATORY STUDY ID

015GSG4300

Polymerase Chain Reaction (PCR) Method Validation for the Detection of Maize Containing Event TC1507

SUMMARY

The objective of this study was to validate a polymerase chain reaction (PCR) method for the detection of maize containing Event TC1507.

Event TC1507 is the product of the biolistic insertion of a 6235 base pair (bp) *Pme* I digestion fragment (PHI 8999A) from plasmid PHP 8999. PHI 8999A contains two genes; (po) *cry*1F and *pat* and the genetic elements necessary for their expression. In transformed maize, the expression products of these genes; Cry1F and phosphinothricin acetyltransferase (PAT), respectively, confer resistance to the European Corn Borer (*Ostrinia nubilalis*) and, tolerance to broad spectrum, non-systemic, non-selective herbicides containing the active ingredient glufosinate-ammonium.

The PCR method was developed by GeneScan Analytics GmbH, GeneScan Europe AG, Engesserstrasse 4b, 79108 Freiburg, Germany; the product is a single 194 base pair (bp) amplicon.

Specificity of the method was determined by performing the method on genomic DNA test substance samples from maize (Events TC1507, *Bt* 176, *Bt* 11, MON 810, T25, GA21, Starlink® and *Bt*-Xtra), potato (New Leaf®), wheat and soybean (Round-up Ready®) and a genomic DNA control substance sample from maize (non-transgenic near isoline of Event TC1507). An approximate 190 bp amplicon was produced from genomic DNA test substance samples obtained from maize containing Event TC1507 *only*. No amplicon was observed with the other test and negative control substance samples.

Sensitivity of the method was determined by: 1) analyzing a serial dilution of genomic DNA test substance from maize Event TC1507 and, 2) analyzing genomic DNA from maize grain reference samples containing 0, 0.1, 0.5, 1 and 5% Event TC1507 (kernel count basis). The method could detect a 1: 10,000 dilution of Event TC1507 genomic DNA, corresponding to approximately 10 genomic copies per PCR reaction. All reference samples containing maize Event TC1507 at a concentration of ≥ 0.1% produced an approximate 190 bp amplicon. No amplicon was observed in the reference sample containing 0% Event TC1507.

Based on the results of this study it is concluded that the PCR method for Event TC1507 is specific, *i.e.*, it could discriminate between (or uniquely identify) a test substance of maize Event TC1507 genomic DNA and those from maize containing other *Bacillus thuringiensis* genes, potato, wheat and soybean and, a control substance of genomic DNA from a non-transgenic near isoline of Event TC1507. The method is also sensitive, *i.e.*, it could detect a reference standard of TC1507 at a level of 0.1% (kernel count basis).

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TOTAL NUMBER OF PAGES: 21

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

Title: Polymerase Chain Reaction (PCR) Method Validation for the Detection of Maize Containing Event TC1507

Information claimed confidential on the basis of its falling within the scope of FIFRA Section 10 (d)(1)(A)(B), or (C) has been removed to a confidential attachment.

Company: Dow AgroSciences, LLC

Company Agent: P. L. Hunst

Title: Regulatory Manager

Signature: ______________________

Date: ______________________

STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Polymerase Chain Reaction (PCR) Method Validation for the Detection of Maize Containing Event TC1507

Study Initiation Date:	02/15/2001	Study Completion Date:	09/06/2001
Experimental Start Date:	02/22/2001	Experimental Termination Date:	04/26/2001

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

This study was not conducted according to Good Laboratory Practice Standards.

P. L. Hunst Sponsor Dow AgroSciences LLC	Date
P. L. Hunst Submitter Dow AgroSciences LLC	Date
I. Lamb Author Pioneer Hi-Bred International, Inc.	Date
A. Nemeth Study Director/Co-Author GeneScan Analytics GmbH	Date

QUALITY ASSURANCE STATEMENT

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NON-GLP STUDY

SIGNATURE PAGE

I. Lamb Author Pioneer Hi-Bred International, Inc.	Date
A. Nemeth Study Director/Co-Author GeneScan Analytics GmbH	Date

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Polymerase Chain Reaction (PCR) Method Validation for the Detection of Maize Containing Event TC1507

ABSTRACT

The objective of this study was to validate a polymerase chain reaction (PCR) method for the detection of maize containing Event TC1507.

Event TC1507 is the product of the biolistic insertion of a 6235 base pair (bp) *Pme* I digestion fragment (PHI 8999A) from plasmid PHP 8999. PHI 8999A contains two genes; (po) *cry*1F and *pat* and the genetic elements necessary for their expression. In transformed maize, the expression products of these genes; Cry1F and phosphinothricin acetyltransferase (PAT), respectively, confer resistance to the European Corn Borer (*Ostrinia nubilalis*) and, tolerance to broad spectrum, non-systemic, non-selective herbicides containing the active ingredient glufosinate-ammonium.

The PCR method was developed by GeneScan Analytics GmbH, GeneScan Europe AG, Engesserstrasse 4b, 79108 Freiburg, Germany; the product is a single 194 base pair (bp) amplicon.

Specificity of the method was determined by performing the method on genomic DNA test substance samples from maize (Events TC1507, *Bt* 176, *Bt* 11, MON 810, T25, GA21, Starlink® and *Bt*-Xtra), potato (New Leaf®), wheat and soybean (Round-up Ready®) and a genomic DNA control substance sample from maize (non-transgenic near isoline of Event TC1507). An approximate 190 bp amplicon was produced from genomic DNA test substance samples obtained from maize containing Event TC1507 *only*. No amplicon was observed with the other test and negative control substance samples.

Sensitivity of the method was determined by: 1) analyzing a serial dilution of genomic DNA test substance from maize Event TC1507 and, 2) analyzing genomic DNA from maize grain reference samples containing 0, 0.1, 0.5, 1 and 5% Event TC1507 (kernel count basis). The

method could detect a 1: 10,000 dilution of Event TC1507 genomic DNA, corresponding to approximately 10 genomic copies per PCR reaction. All reference samples containing maize Event TC1507 at a concentration of $\geq$ 0.1% produced an approximate 190 bp amplicon. No amplicon was observed in the reference sample containing 0% Event TC1507.

Based on the results of this study it is concluded that the PCR method for Event 1507 is specific, *i.e.*, it could discriminate between (or uniquely identify) a test substance of Event TC1507 genomic DNA and those from maize containing other *Bacillus thuringiensis* genes, potato, wheat and soybean and, a control substance of genomic DNA from a non-transgenic near isoline of Event TC1507. The method is also sensitive, *i.e.*, it could detect a reference standard of TC 1507 at a level of 0.1% (kernel count basis).

INTRODUCTION

Maize (*Zea mays*) Event TC1507 is the product of the biolistic insertion of a 6235 base pair (bp) *Pme* I digestion fragment (PHI 8999A) from plasmid PHP 8999. PHI 8999A contains two genes; (po) *cry*1F and *pat* and the genetic elements necessary for their expression. In transformed maize, the expression products of these genes; Cry1F and phosphinothricin acetyltransferase (PAT), respectively, confer resistance to the European Corn Borer (*Ostrinia nubilalis*) and, tolerance to broad spectrum, non-systemic, non-selective herbicides containing the active ingredient glufosinate-ammonium.

The objective of this study was to validate a polymerase chain reaction (PCR) method for the detection of maize containing Event TC1507 developed by GeneScan Analytics GmbH, GeneScan Europe AG, Engesserstrasse 4b, 79108 Freiburg, Germany.

LABORATORY PHASE

Test Substances

See Table 1.

Control Substance

Identity:	Genomic DNA prepared from maize kernels not containing Event TC1507 (*i.e.*, non-transgenic near isoline of test substance containing Event TC1507).
Lot number:	Non-GM-PHI1
Source:	Pioneer Hi-Bred International, Inc.

Storage conditions: -20°C

PCR Method Specificity Evaluation

Specificity of the PCR method for Event TC1507 was evaluated by performing the method with the following test substances as template DNA: genomic DNA from maize containing Event TC1507, *Bt* 176, *Bt* 11, MON 810, T25, Starlink®, GA 21 and *Bt*-Xtra. Genomic DNA from New Leaf® potato, wheat and Round-up Ready® soybean was also evaluated. The control substance employed was genomic DNA from the non-transgenic near isoline of Event TC1507. A PCR negative control sample (no DNA template) was also evaluated.

Maize (flour prepared from kernels), potato, wheat and soybean tissues were extracted and analyzed in duplicate, as described in Appendix 1.

PCR Method Sensitivity Evaluation

Sensitivity of the PCR method for Event TC1507 was evaluated by:

1) Analyzing a serial dilution of the test substance for Event TC1507 (Cry1F-PHI). The non-diluted (stock) sample contained 45 ng genomic DNA/µl. The dilution series of the stock was as follows: 1:10, 1:100, 1:1000, 1:10,000, 1: 100,000 and 1: 1,000,000. The PCR method was performed in duplicate on the dilution series samples as described in Appendix 1. A PCR negative control sample (no DNA template) was also evaluated.

2) Analyzing genomic DNA from maize reference standards (kernel flour) containing 0%, 0.1%, 0.5%, 1.0% and 5% Event TC1507 (Cry1F-PHI; kernel count basis). Each reference standard was extracted and analyzed in duplicate as described in Appendix 1. Extraction and PCR negative control samples (no sample or DNA template, respectively) were also evaluated.

RESULTS

PCR Method Specificity Evaluation

An approximate 190 bp amplicon was produced from genomic DNA test substance samples from maize containing Event TC1507 *only*. No amplicon was observed with the other test and negative control substance samples, or the PCR negative control sample (see Figure 1).

PCR Method Sensitivity Evaluation

The method could detect a 1: 10,000 dilution of Event TC1507 genomic DNA, corresponding to approximately 10 genomic copies per PCR reaction. No amplicon was observed with the 1: 100,000 and 1: 1,000,000 dilutions or the PCR negative control sample (see Figure 2).

All reference samples containing maize Event TC1507 at a concentration of $\geq$ 0.1% produced an approximate 190 bp amplicon. No amplicon was observed in the reference sample containing 0% Event TC1507 or the extraction and PCR negative control samples (see Figure 3).

CONCLUSIONS

Under the conditions of this study:

The PCR method for maize Event TC 1507 was shown to be specific, *i.e.*, it could discriminate between (or uniquely identify) a test substance containing Event TC1507 and those from maize containing other *Bacillus thuringiensis* genes, potato, wheat and soybean and, a control substance not containing Event TC 1507 (non-transgenic near isoline of maize Event TC1507).

The PCR method for maize Event TC1507 was shown to be sensitive; *i.e.*, it could detect a reference standard of Event TC1507 at a level of 0.1% (kernel count basis).

Table 1. Test Substances

Test Substance Identity*	**Lot Number**	**Source**	**Storage Conditions (°C)**
Event TC1507 (maize)	Cry1F - PHI	Pioneer Hi-Bred International, Inc.	-20
Event TC1507 (maize)	Cry1F - DAS	DowAgroSciences, LLC	-20
Event *Bt* 176 (maize)	Bt 176D/02-01	GeneScan Analytics GmbH	-20
Event *Bt* 11 (maize)	Bt 11D/03-01	GeneScan Analytics GmbH	-20
Event MON810 (maize)	MON810D/01-01	GeneScan Analytics GmbH	-20
Event T25 (maize)	T25D/01-01	GeneScan Analytics GmbH	-20
Starlink®[1] (maize)	StarlinkD/03-01	GeneScan Analytics GmbH	-20
Event GA21 (maize)	GA21D/02-01	GeneScan Analytics GmbH	-20
Bt-Xtra®[2] (maize)	Bt-XtraD/02-01	GeneScan Analytics GmbH	-20
New Leaf®[3]Potato	New LeafD/02-01	GeneScan Analytics GmbH	-20
Wheat	WheatD/Reg-01	GeneScan Analytics GmbH	-20
Roundup Ready®[4] Soybean	RRSoyD/05-01	GeneScan Analytics GmbH	-20

* Refers to genomic DNA in all cases

[1]Registered trademark of Aventis, Inc., [2]Registered trademark of DeKalb, Inc., [3,4] Registered trademark of Monsanto Company, Inc.

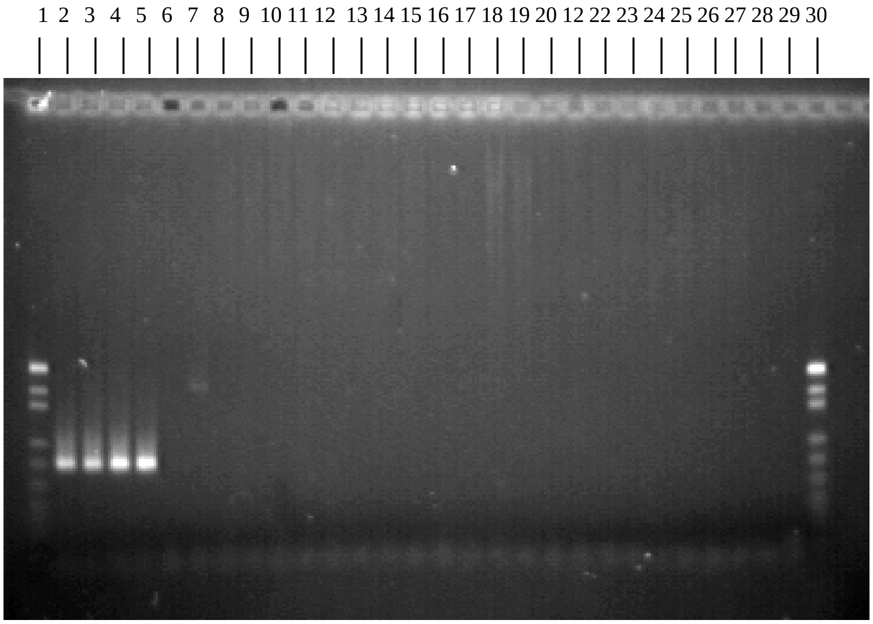

Figure 1: Maize Event TC1507 PCR Method: Specificity Evaluation. 2.5% agarose gel stained with ethidium bromide. 5 µl of PCR product loaded per lane.

Lane	**Sample**
1	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp (4 µl puc19/*Hpa* II [50 ng/µl])
2-3	Event TC1507 (Cry1F-PHI)
4-5	Event TC1507 (Cry1F-DAS)
6-7	Non-transgenic near isoline of Event TC1507
8-9	Event *Bt* 176
10-11	Event *Bt* 11
12-13	Event MON 810
14-15	Event T25
16-17	Starlink®
18-19	Event GA21
20-21	*Bt*-Xtra
22-23	New Leaf® potato
24-25	Wheat
26-27	Roundup Ready® soybean
28-29	PCR negative control
30	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp (4 µl puc19/*Hpa* II [50 ng/µl])

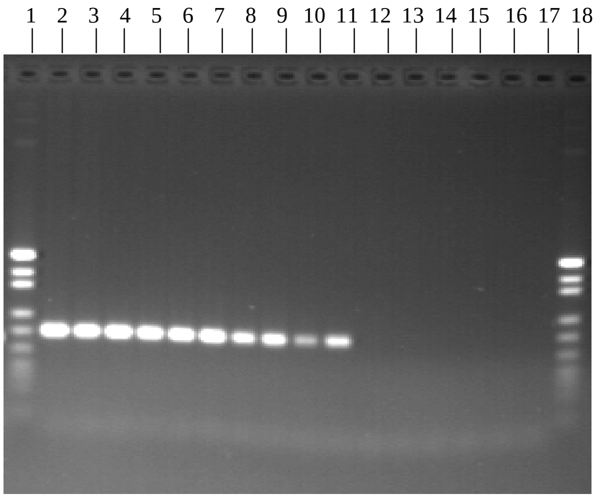

Figure 2: Maize Event TC1507 PCR Method: Sensitivity Evaluation. 2.5 % agarose gel stained with ethidium bromide. 5 µl of PCR product loaded per lane.

Lane	**Sample**
1	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp (4 µl puc19/*Hpa* II [50 ng/µl])
2-3	Undiluted stock solution of Event TC1507 (Cry1F-PHI) genomic DNA
4-5	1:10 dilution of stock solution
6-7	1:100 dilution of stock solution
8-9	1:1,000 dilution of stock solution
10-11	1:10,000 dilution of stock solution
12-13	1:100,000 dilution of stock solution
14-15	1:1,000,000 dilution of stock solution
16-17	PCR negative control
18	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp (4 µl puc19/*Hpa* II [50 ng/µl])

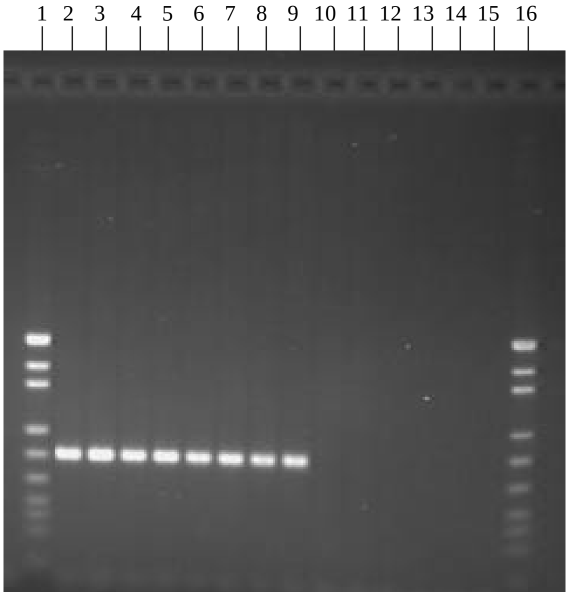

Figure 3: Maize Event TC1507 PCR Method: Sensitivity Evaluation. 2.5% agarose gel stained with ethidium bromide. 5µl of PCR product loaded per lane.

Lane	Sample
1	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp (4 µl puc19/*Hpa* II [50 ng/µl])
2-3	Event TC1507 (Cry1F-PHI): 5% reference standard
4-5	Event TC1507 (Cry1F-PHI): 1% reference standard
6-7	Event TC1507 (Cry1F-PHI): 0.5% reference standard
8-9	Event TC1507 (Cry1F-PHI): 0.1% reference standard
10-11	Event TC1507 (Cry1F-PHI): 0% reference standard
12-13	Extraction negative control
14-15	PCR negative control
16	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp (4 µl puc19/*Hpa* II [50 ng/µl])

APPENDIX 1—PCR Detection Method for Maize Containing Event TC1507

METHOD

Scope

This method is designed for the analysis of maize grain for the presence of Event TC1507.

Principle

This method may be employed in one of two ways:

1) To detect the absence and presence of Event TC1507 in maize grain. This test may also be used to confirm the specificity of the method.

2) To detect the absence or presence of Event TC1507 in reference standards containing fractions of maize grain containing Event TC1507. This test may also be used to confirm the sensitivity of the method.

It is recommended that the specificity and sensitivity of the PCR Detection method for Event TC1507 is confirmed prior to use in the analysis of commercial maize grain samples.

Equipment

1. GeneAmp PCR System 9700, Applied Biosystems, Weiterstadt, Germany or equivalent.

2. Water purification system, Milli-Q UV, Millipore Corporation, Bedford, MA 01730 or equivalent.

3. Balance, Sartorius, Fisher Scientific or equivalent.

4. Eppendorf pipettes (10-1000), Fisher Scientific or equivalent.

5. Centrifuge, Heraeus Biofuge, Fisher Scientific or equivalent.

6. Oven, Heraeus Oven, Fisher Scientific or equivalent.

7. Gel Electrophoresis Unit, Biometra Agagel, Göettingen, Germany or equivalent.

8. Microfluorimeter, Pharmacia Gene Quant, Freiburg, Germany or equivalent.

Materials

1. CTAB buffer (1.4 M NaCl, 2% CTAB, 100 mM Tris, 15 mM EDTA). Adjust to pH 8.0 with 25% HCl.

2. DNeasy Plant Mini Kit, Qiagen, Hilden, Germany or equivalent. Qiagen kit contains buffer AE, buffer AP2, buffer AP3, buffer AW, RNase A, DNeasy spin columns and 2.0 ml microcentrifuge collection tubes.

3. Ethanol (96-100% w/w), Fluka, Taufkirchen, Germany or equivalent.

4. Proteinase K, Merck, Bruchsal, Germany or equivalent.

5. AmpliTaq Gold DNA Polymerase (5U/µl) with GeneAmp 10x PCR Gold buffer (with $MgCl_2$), Applied Biosystems, Weiterstadt, Germany or equivalent.

6. dNTP's, Hybaid, Heidelberg, Germany or equivalent.

7. Forward and reverse primers (10 µM; Cry1F-f and Cry1F-r). See Confidential Attachment.

8. Agarose, Roth, Karlsruhe, Germany or equivalent

Event TC1507 Maize Grain Test and Control Substances

Control Maize Grain (Kernels). This may be non-transgenic or transgenic maize grain not containing Event TC1507.

Event TC1507 Maize Grain (Kernels). This should be transgenic maize grain containing Event TC1507.

PROCEDURES

DNA Extraction, Purification and Quantitation

Undertake the following steps in consecutive order:

1. Combine 2 grams of ground maize grain sample with 10 ml CTAB buffer and 30 µl of proteinase K (20 mg/ml) in a centrifuge tube. Mix well using a vortex mixer.

2. Prepare extraction buffer negative control samples. Combine 10 ml CTAB buffer and 30 µl of proteinase K (20 mg/ml) in a centrifuge tube. Mix well using a vortex mixer.

3. Incubate overnight (minimum of 2 hours) at 60°C in a waterbath or oven, with agitation.

4. Centrifuge for 2-5 minutes at ≥ 3,000 x g.

5. Transfer 1 ml of supernatant into a 1.5 ml tube containing 5 µl of RNase A solution from DNeasy Plant Mini Kit. Incubate for a minimum of 15 minutes at 60°C.

6. Centrifuge for a minimum of 1 minute at 20,000 x g.

7. Combine 400 µl of the supernatant with 130 µl of buffer AP2 from DNeasy Plant Mini Kit in 1.5 ml tube. Incubate for 5 minutes at 4°C. Centrifuge for 5 minutes at 20,000 x g.

8. Combine 400 µl of the supernatant with 600 µl of buffer AP3 from DNeasy Plant Mini Kit in a 1.5 ml tube. Mix well using pipette.

9. Load a DNeasy spin column with 600 μl of the supernatant/buffer AP3 mix and centrifuge for 1 minute at 6,000 x g.

10. Transfer the DNeasy spin column to a new 2.0 ml collection tube. Add 500 μl of buffer AW from DNeasy Plant Mini Kit to DNeasy spin column. Centrifuge for 1 minute at 6,000 x g.

11. Discard the buffer and place DNeasy spin column back into the 2.0 ml collection tube. Add 500 μl of buffer AW to the DNeasy spin column. Centrifuge for 2 minutes at 20,000 x g.

12. Place DNeasy spin column into 2.0 ml collection tube. Add 2 x 50 μl preheated buffer AE (60°C) from DNeasy Plant Mini Kit and incubate for 1-5 minutes.

13. Centrifuge for 1 minute at 6,000 x g.

14. Determine the DNA concentration using a microfluorimeter or 1.5% agarose gel with DNA concentration reference standard(s).

PCR Conditions

Undertake the following steps in consecutive order:

1. Prepare the following reaction mixture, in duplicate:

Component	**Volume (μl)**
Water	13.34
10x PCR buffer	2.50
dNTP (4mM each)	1.00
Forward primer (10 μM; Cry1F-f)*	1.50

Reverse primer (10µM; Cry1F-r)*	1.50
Ampli Taq Gold (5U/ µl)	0.16
Genomic DNA template	5.00
Total reaction volume	**25**

*See Confidential Attachment for details regarding forward and reverse primers.

2. The PCR is performed using a GeneAmp PCR System 9700, or equivalent, with the following cycle conditions:

Cycle Element	**Temperature (°C)**	**Time**	**Number of Cycles**
Initial Denaturation	95	10 minutes	1
Denaturation	95	25 seconds	45
Annealing	64	30 seconds	
Elongation	72	45 seconds	
Final Elongation	72	7 minutes	1

3. Analyze a 5 µl aliquot of the PCR mixture on a 2.5% agarose gel. Samples containing Event TC1507 genomic DNA will result in a 194 bp amplicon. Samples that do not contain Event TC1507 genomic DNA will not result in a PCR product.

CONFIDENTIAL ATTACHMENT

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FIGURES

This Confidential Attachment contains information regarding the primers used in the PCR Method for Maize Event TC1507.

1. Forward Primer (Cry1F-f)

   5'- gac ctg cag aag ctt cgg t - 3'

2. Reverse Primer (Cry1F-r)

   5'- cga ata gaa aat act gca ctg caa - 3'

3. Length of Amplicon

   194 bp. See Figure 1.

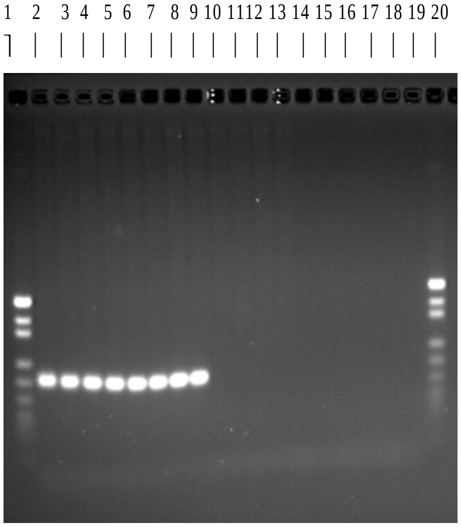

Figure 1. PCR Product for Maize Event TC1507

Lane	**Sample**
1	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp
2-5	Maize Event TC1507 from Pioneer Hi-Bred International, Inc
6-9	Maize Event TC1507 from Dow AgroSciences, LLC
10-13	Non-transgenic near isoline of Maize Event TC1507
14-17	Extraction negative control
18-19	PCR reaction negative control
20	DNA ladder (descending order on gel): 501/498, 409, 325, 242, 190, 147, 110, 67, 34 and 26 bp

4. Sequence Confirmation of Amplicon

The forward and reverse primers were developed based on sequence information derived from the 3' end of the inserted *Pme* I fragment (PHI8999A) in Event TC1507. Immediately distal (3') to the *Pme* I fragment (PHI8999A) in Event TC1507 is an inverted repeat region of the *cry* 1F gene. The amplicon spans the border between these two regions (3' end of *Pme* I fragment and inverted repeat region of the *cry* 1F gene) and provides a unique identification method for Event TC1507. Double-strand sequencing of the amplicon (performed by Dr. G. Igloi, Institute for Biology III, University of Freiburg, Germany) derived from maize samples containing Event TC1507 confirmed the sequence for this region in Event TC1507 (see Figure 2).

TC1507-PHI	GACCTGCAGA AGCTTCGGTC CGGCGCGCCT CTAGTTGAAG ACACGTTCAT	
TC1507-DAS	GACCTGCAGA AGCTTCGGTC CGGCGCGCCT CTAGTTGAAG ACACGTTCAT	
TC1507 seq.	***GACCTGCAGA AGCTTCGGTC CGGCGCGCCT CTAGTTGAAG ACACGTTCAT***	***110***
TC1507-PHI	GTCTTCATCG TAAGAAGACA CTCAGTAGTC TTCGGCCAGA ATGGCCTAAC	
TC1507-DAS	GTCTTCATCG TAAGAAGACA CTCAGTAGTC TTCGGCCAGA ATGGCCTAAC	
TC1507 seq.	***GTCTTCATCG TAAGAAGACA CTCAGTAGTC TTCGGCCAGA ATGGCCTAAC***	***160***
TC1507-PHI	TCAAGGCCCT CACTCCGCTT GATCTTGGCA AAGATATTTG ACGCATTTAT	
TC1507-DAS	TCAAGGCCCT CACTCCGCTT GATCTTGGCA AAGATATTTG ACGCATTTAT	
TC1507 seq.	***TCAAGGCCCT CACTCCGCTT GATCTTGGCA AAGATATTTG NCGCATTTAT***	***210***
TC1507-PHI	TAGTATGTGT TAATTTTCAT TTGCAGTGCA GTATTTTCTA TTCG	
TC1507-DAS	TAGTATGTGT TAATTTTCAT TTGCAGTGCA GTATTTTCTA TTCG	
TC1507 seq.	***TAGTATGTGT TAATTTTCAT TTGCAGTGCA GTATTTTCTA TTCG***	***254***

Figure 2: Alignment of the sequences of PCR products amplified from DNA samples obtained from maize provided by Pioneer Hi-Bred International, Inc. (TC1507-PHI) and Dow AgroSciences, LLC (TC1507-DAS) with the sequence from Event TC1507 (nucleotide 61-254). Forward and reverse primer binding sites are underlined.