P1 1201531

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

August 05, 2004

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/467,772*
FILING DATE: *May 02, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/13538*

RECEIVED
09 AUG 2004
WIPO PCT

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

M. Tarver

M. TARVER
Certifying Officer

PRIORITY DOCUMENT
SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH
RULE 17.1(a) OR (b)

Inari Exhibit 1004
Inari v. Corteva

05-05-03 60467772.0502

PTO/SB/16 (10-01) 44A/
Approved for use through 10/31/2002. OMB 0651-0032
Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

Express Mail Label No. EL870385389 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Mary E.	Hartnett Locke	Mickleton, NJ

☐ *Additional inventors are being named on the ____ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

Corn Event TC1507 and Methods for Detection Thereof

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☒ Customer Number 27310 → *Place Customer Number Bar Code Label here*

OR *Type Customer Number here*

☐ Firm *or* Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	

ENCLOSED APPLICATION PARTS *(check all that apply)*

☒ Specification *Number of Pages* 51 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 1 ☒ Other (specify) Sequence Listing and Statement regarding Sequence Listing

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☐ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

FILING FEE AMOUNT ($)

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 16-1852 — 160.00

☐ Payment by credit card. Form PTO-2038 is attached.

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are: ____.

Respectfully submitted,

SIGNATURE Amy L. Cigan Date 5/2/03

TYPED or PRINTED NAME Amy L. Cigan REGISTRATION NO. *(if appropriate)* 45,494

TELEPHONE 515-248-4896 Docket Number: 1523P

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Certificate of Mailing under 37 CFR 1.10

Express Mailing Label Number EL870385389US

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as "Express Mail Post Office to Addressee" Service in an envelope addressed to:

Mail Stop Provisional Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

on May 2, 2003.

Signature

Tracy Westphal
Typed or printed name of person signing Certificate

Note: Each paper must have its own certificate of mailing, or this certificate must identify each submitted paper.

1. Provisional Application for Patent Cover Sheet (1 orig + 1 copy)
2. Specification (1 copy)
3. Fig. 1 (1 copy)
4. Application Data Sheet (1 copy)
5. Statement to Support Filing and Submission in Accordance with 37 CFR 1.821 through 1.825 (1 copy)
6. Sequence Listing (1 paper copy + 1 diskette)

Copy provided by USPTO from the PACR Image Database on 07/26/2004

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Mary E. Hartnett Locke
Application Number	:	
Filing Date	:	May 2, 2003
Title	:	Corn Event TC1507 and Methods for Detection Thereof
Group/Art Unit	:	
Examiner	:	
Attorney Docket Number	:	1523P

Mail Stop Sequence
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

STATEMENT TO SUPPORT FILING AND SUBMISSION IN ACCORDANCE WITH 37 CFR 1.821 THROUGH 1.825

☒ I hereby state that the contents of the paper and computer readable copies of the Sequence Listing, submitted in accordance with 37 CFR 1.821(c) and (e), respectively, are the same.

☐ I hereby state that the submission filed in accordance with 37 CFR 1.821(g) does not include new matter.

☐ I hereby state that the submission filed in accordance with 37 CFR 1.821(h) does not include new matter or go beyond the disclosure in the international application as filed.

☐ I hereby state that the amendments, made in accordance with 37 CFR 1.825(a), included in the substitute sheet(s) of the Sequence Listing are supported in the application, as filed, at pages _____. I hereby state that the substitute sheet(s) of the Sequence Listing does (do) not include new matter.

☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with 37 CFR 1.825(b), is the same as the amended Sequence Listing.

Copy provided by USPTO from the PACR Image Database on 07/20/2004

☐ I hereby state that the substitute copy of the computer readable form, submitted in accordance with 37 CFR 1.825(d), is identical to that originally filed.

Respectfully submitted,

Amy L. Cigan
Agent for Applicant(s)
Registration No. 45,494

PIONEER HI-BRED INTERNATIONAL, INC.
Corporate Intellectual Property
7100 N.W. 62nd Avenue
P.O. Box 1000
Johnston, Iowa 50131-1000
Phone: (515) 248-4896
Facsimile: (515) 334-6883

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Application Data Sheet

Application Information

Application Type::	Provisional
Subject Matter::	
Suggested Classification::	
Suggested Group Art Unit::	
CD-ROM or CD-R?::	
Number of CD disks::	
Number of copies of CDs::	
Sequence submission?::	paper
Computer Readable Form (CRF)?::	Yes
Number of copies of CRF::	01
Title::	Corn Event TC1507 and Methods for Detection Thereof
Attorney Docket Number::	1523P
Request for Early Publication?::	
Request for Non-Publication?::	
Suggested Drawing Figure::	
Total Drawing Sheets::	1
Small Entity::	
Petition included?::	
Secrecy Order in Parent Appl.?::	

Applicant Information:

Applicant Authority type::	Inventor
Primary Citizenship Country::	US
Status::	Full Capacity
Given Name::	Mary
Middle Name::	E.

1 Initial 5/1/2003

Copy provided by USPTO from the PACR Image Database on 07/20/2004

Family Name::	Hartnett Locke
City of Residence::	Mickleton
State or Province of Residence::	NJ
Country of Residence::	US
Street of mailing address::	31 Millwood Drive
City of mailing address::	Mickleton
State or Province of mailing address::	NJ
Postal or Zip Code of mailing address::	08056

Correspondence Information

Correspondence Customer Number:: 27310

Representative Information

Representative Customer Number:: 27310

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::

Assignee Information

Assignee Name:: Pioneer Hi-Bred International, Inc.

[redacted]

Copy provided by USPTO from the PACR Image Database on 07/26/2004

CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

FIELD OF THE INVENTION

The present invention relates to the field of plant molecular biology, specifically the invention relates to a DNA construct for conferring insect resistance to a plant. The invention more specifically relates to an insect resistant corn plant TC1507 and to assays for detecting the presence of corn plant TC1507 DNA in a sample and compositions thereof.

BACKGROUND OF THE INVENTION

This invention relates to the insect resistant corn (*Zea mays*) plant TC1507, also referred to as maize line TC1507 or maize event TC1507, and to the DNA plant expression construct of corn plant TC1507 and the detection of the transgene/flanking insertion region in corn plant TC1507 and progeny thereof.

Corn is an important crop and is a primary food source in many areas of the world. Damage caused by insect pests is a major factor in the loss of the world's corn crops, despite the use of protective measures such as chemical pesticides. In view of this, insect resistance has been genetically engineered into crops such as corn in order to control insect damage and to reduce the need for traditional chemical pesticides. One group of genes which have been utilized for the production of transgenic insect resistant crops are the delta-endotoxins from *Bacillus thuringiensis (B.t.)*. Delta-endotoxins have been successfully expressed in crop plants such as cotton, potatoes, rice, sunflower, as well as corn, and have proven to provide excellent control over insect pests. (Perlak, F.J *et al.* (1990) *Bio/Technology* 8, 939-943; Perlak, F.J. *et al.* (1993) *Plant Mol. Biol.* 22: 313-321; Fujimoto H. *et al.* (1993) *Bio/Technology* 11: 1151-1155; Tu *et al.* (2000) *Nature Biotechnology* 18:1101-1104; PCT publication number WO 01/13731; and Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, Fort Collins, CO).

The expression of foreign genes in plants is known to be influenced by their location in the plant genome, perhaps due to chromatin structure (e.g., heterochromatin) or the proximity of transcriptional regulatory elements (e.g., enhancers) close to the integration site (Weising *et al.*, *Ann. Rev. Genet* 22:421-477, 1988). At the same time the presence of the transgene at different locations in the genome will influence the overall phenotype of the plant in different ways. For this reason, it is often necessary to screen a large number of events in order to

Copy provided by USPTO from the PACR Image Database on 07/20/2004

identify an event characterized by optimal expression of an introduced gene of interest. For example, it has been observed in plants and in other organisms that there may be a wide variation in levels of expression of an introduced gene among events. There may also be differences in spatial or temporal patterns of expression, for example, differences in the relative expression of a transgene in various plant tissues, that may not correspond to the patterns expected from transcriptional regulatory elements present in the introduced gene construct. For this reason, it is common to produce hundreds to thousands of different events and screen those events for a single event that has desired transgene expression levels and patterns for commercial purposes. An event that has desired levels or patterns of transgene expression is useful for introgressing the transgene into other genetic backgrounds by sexual outcrossing using conventional breeding methods. Progeny of such crosses maintain the transgene expression characteristics of the original transformant. This strategy is used to ensure reliable gene expression in a number of varieties that are well adapted to local growing conditions.

It would be advantageous to be able to detect the presence of a particular event in order to determine whether progeny of a sexual cross contain a transgene of interest. In addition, a method for detecting a particular event would be helpful for complying with regulations requiring the pre-market approval and labeling of foods derived from recombinant crop plants, for example, or for use in environmental monitoring, monitoring traits in crops in the field, or monitoring products derived from a crop harvest, as well as for use in ensuring compliance of parties subject to regulatory or contractual terms.

It is possible to detect the presence of a transgene by any nucleic acid detection method known in the art including, but not limited to, the polymerase chain reaction (PCR) or DNA hybridization using nucleic acid probes. These detection methods generally focus on frequently used genetic elements, such as promoters, terminators, marker genes, etc., because for many DNA constructs, the coding region is interchangeable. As a result, such methods may not be useful for discriminating between different events, particularly those produced using the same DNA construct or very similar constructs unless the DNA sequence of the flanking DNA adjacent to the inserted heterologous DNA is known. For example, an event-specific PCR assay is described in U.S. Patent No. 6,395,485 for the detection of elite event GAT-ZM1. Accordingly, it would be desirable to have a simple and discriminative method for the identification of event TC1507.

Copy provided by USPTO from the PACR Image Database on 07/20/2004

SUMMARY OF THE INVENTION

This invention relates preferably to methods for producing and selecting an insect resistant monocot crop plant. More specifically, a DNA construct is provided that when expressed in plant cells and plants confers resistance to insects. According to one aspect of the invention, a DNA construct, capable of introduction into and replication in a host cell, is provided that when expressed in plant cells and plants confers insect resistance to the plant cells and plants. The DNA construct is comprised of a DNA molecule named PHI8999A and it includes two transgene expression cassettes. The first expression cassette comprises a DNA molecule which includes the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689 and Christensen and Quail (1996) *Transgenic Res.* 5:213-218) operably connected to a DNA molecule encoding a *B.t.* δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350). The second transgene expression cassette of the DNA construct comprises a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59) operably connected to a DNA molecule encoding a phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene* 70: 25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59). Plants containing the DNA construct are also provided.

According to another aspect of the invention, compositions and methods are provided for identifying a novel corn plant designated TC1507, which methods are based on primers or probes which specifically recognize the 5' and/or 3' flanking sequence of TC1507. DNA molecules are provided that comprise primer sequences that when utilized in a PCR reaction will produce amplicons unique to the transgenic event TC1507. These molecules may be selected from the group consisting of:

5'-GTAGTACTATAGATTATATTATTCGTAGAG-3' (SEQ ID NO: 1);

5'-GCCATACAGAACTCAAAATCTTTTCCGGAG-3' (SEQ ID NO: 2);

5'- CTTCAAACAAGTGTGACAAA –3' (SEQ ID NO: 23);

5'-TGTGGTGTTTGTGGCTCTGTCCTAA-3' (SEQ ID NO: 3);

Copy provided by USPTO from the PACR Image Database on 07/26/2004

5'-AGCACCTTTTCATTCTTTCATATAC-3' (SEQ ID NO: 4);
5'-GACCTCCCCA CAGGCATGAT TGATC-3' (SEQ ID NO: 5);
and complements thereof. The corn plant and seed comprising these molecules is an aspect of this invention. Further, kits utilizing these primer sequences for the identification of the TC1507 event are provided.

An additional aspect of the invention relates to the specific flanking sequences of TC1507 described herein, which can be used to develop specific identification methods for TC1507 in biological samples. More particularly, the invention relates to the 5' and/or 3' flanking regions of TC1507, SEQ ID NO:21 and SEQ ID NO:22, respectively, which can be used for the development of specific primers and probes. The invention further relates to identification methods for the presence of TC1507 in biological samples based on the use of such specific primers or probes.

According to another aspect of the invention, methods of detecting the presence of DNA corresponding to the corn event TC1507 in a sample are provided. Such methods comprise: (a) contacting the sample comprising DNA with a DNA primer set, that when used in a nucleic acid amplification reaction with genomic DNA extracted from corn event TC1507 produces an amplicon that is diagnostic for corn event TC1507; (b) performing a nucleic acid amplification reaction, thereby producing the amplicon; and (c) detecting the amplicon.

DNA molecules that comprise the novel transgene/flanking insertion region, SEQ ID NO: 26 and SEQ ID NO: 27 and are homologous or complementary to SEQ ID NO: 26 and SEQ ID NO: 27 are an aspect of this invention.

DNA sequences that comprise the novel transgene/flanking insertion region, SEQ ID NO:26 are an aspect of this invention. DNA sequences that comprise a sufficient length of polynucleotides of transgene insert sequence and a sufficient length of polynucleotides of maize genomic and/or flanking sequence from maize plant TC1507 of SEQ ID NO:26 that are useful as primer sequences for the production of an amplicon product diagnostic for maize plant TC1507 are included.

In addition, DNA sequences that comprise the novel transgene/flanking insertion region, SEQ ID NO:27 are provided. DNA sequences that comprise a sufficient length of polynucleotides of transgene insert sequence and a sufficient length of polynucleotides of maize genomic and/or flanking sequence from maize plant TC1507 of SEQ ID NO:27 that are

Copy provided by USPTO from the PACR Image Database on 07/26/2004

useful as primer sequences for the production of an amplicon product diagnostic for maize plant TC1507 are included.

According to another aspect of the invention, the DNA sequences that comprise at least 11 or more nucleotides of the transgene portion of the DNA sequence of SEQ ID NO:26 or complements thereof, and a similar length of 5' flanking maize DNA sequence of SEQ ID NO:26 or complements thereof are useful as DNA primers in DNA amplification methods. The amplicons produced using these primers are diagnostic for maize event TC1507. Therefore, the invention also includes the amplicons produced by DNA primers homologous or complementary to SEQ ID NO:26.

According to another aspect of the invention, the DNA sequences that comprise at least 11 or more nucleotides of the transgene portion of the DNA sequence of SEQ ID NO:27 or complements thereof, and a similar length of 3' flanking maize DNA sequence of SEQ ID NO:27 or complements thereof are useful as DNA primers in DNA amplification methods. The amplicons produced using these primers are diagnostic for maize event TC1507. Therefore, the invention also includes the amplicons produced by DNA primers homologous or complementary to SEQ ID NO:27.

More specifically, a pair of DNA molecules comprising a DNA primer set, wherein the DNA molecules are identified as SEQ ID NO: 1 or complements thereof and SEQ ID NO: 2 or complements thereof; SEQ ID NO: 2 or complements thereof and SEQ ID NO: 23 or complements thereof; SEQ ID NO: 3 or complements thereof and SEQ ID NO: 5 or complements thereof; SEQ ID NO: 4 or complements thereof and SEQ ID NO: 5 or complements thereof are aspects of the invention.

Further aspects of the invention include the amplicon comprising the DNA molecules of SEQ ID NO: 1 and SEQ ID NO: 2; the amplicon comprising the DNA molecules of SEQ ID NO: 2 and SEQ ID NO: 23; the amplicon comprising the DNA molecules of SEQ ID NO: 3 and SEQ ID NO: 5; and the amplicon comprising the DNA molecules of SEQ ID NO: 4 and SEQ ID NO: 5.

According to another aspect of the invention, methods of detecting the presence of a DNA molecule corresponding to the TC1507 event in a sample, such methods comprising: (a) contacting the sample comprising DNA extracted from a corn plant with a DNA probe, molecule that hybridizes under stringent hybridization conditions with DNA extracted from corn event TC1507 and does not hybridize under the stringent hybridization conditions with a

Copy provided by USPTO from the PACR Image Database on 07/26/2004

control corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA. More specifically, a method for detecting the presence of a DNA molecule corresponding to the TC1507 event in a sample, such methods, consisting of (a) contacting the sample comprising DNA extracted from a corn plant with a DNA probe molecule that consists of sequences that are unique to the event, e.g. junction sequences, wherein said DNA probe molecule hybridizes under stringent hybridization conditions with DNA extracted from corn event TC1507 and does not hybridize under the stringent hybridization conditions with a control corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA.

In addition, a kit and methods for identifying event TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24 are provided.

DNA molecules are provided that comprise at least one junction sequence of TC1507 selected from the group consisting of SEQ ID NO:45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; wherein a junction sequence spans the junction between heterologous DNA inserted into the genome and the DNA from the corn cell flanking the insertion site, i.e. flanking DNA, and is diagnostic for the TC1507 event.

According to another aspect of the invention, methods of producing an insect resistant corn plant that comprise the steps of: (a) sexually crossing a first parental corn line comprising the expression cassettes of the present invention, which confers resistance to insects, and a second parental corn line that lacks insect resistance, thereby producing a plurality of progeny plants; and (b) selecting a progeny plant that is insect resistant. Such methods may optionally comprise the further step of back-crossing the progeny plant to the second parental corn line to producing a true-breeding corn plant that is insect resistant.

The present invention provides a method of producing a corn plant that is resistant to insects comprising transforming a corn cell with the DNA construct PHI8999A (SEQ ID NO:25), growing the transformed corn cell into a corn plant, selecting the corn plant that shows resistance to insects, and further growing the corn plant into a fertile corn plant. The fertile corn plant can be self pollinated or crossed with compatible corn varieties to produce insect resistant progeny.

The invention further relates to a DNA detection kit for identifying maize event TC1507 in biological samples. Preferably the kit of the invention comprises a first primer

Copy provided by USPTO from the PACR Image Database on 07/26/2004

which specifically recognizes the 5' or 3' flanking region of TC1507, and a second primer which specifically recognizes a sequence within the foreign DNA of TC1507, or within the flanking DNA, for use in a PCR identification protocol. The invention also relates to a kit for identifying event TC1507 in biological samples, which kit comprises a specific probe having a sequence which corresponds or is complementary to, a sequence having between 80% and 100% sequence identity with a specific region of event TC1507. Preferably the sequence of the probe corresponds to a specific region comprising part of the 5' or 3' flanking region of event TC1507.

The methods and kits encompassed by the present invention can be used for different purposes such as, but not limited to the following: to identify event TC1507 in plants, plant material or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material; additionally or alternatively, the methods and kits of the present invention can be used to identify transgenic plant material for purposes of segregation between transgenic and non-transgenic material; additionally or alternatively, the methods and kits of the present invention can be used to determine the quality of plant material comprising maize event TC1507. The kits may also contain the reagents and materials necessary for the performance of the detection method.

This invention further relates to the TC1507 corn plant or its parts, including, but not limited to, pollen, ovules, vegetative cells, the nuclei of pollen cells, and the nuclei of egg cells of the corn plant TC1507 and the progeny derived thereof. The corn plant and seed TC1507 from which the DNA primer molecules of the present invention provide a specific amplicon product is an aspect of the invention.

The foregoing and other aspects of the invention will become more apparent from the following detailed description and accompanying drawing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Linear map showing the transgenic insert PHI8999A, as well as the sequences flanking the transgenic insert.

DETAILED DESCRIPTION

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al., Glossary of Genetics: Classical and Molecular, 5th edition,* Springer-Verlag; New York, 1991; and Lewin, *Genes V,* Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at 37 CFR 1.822 is used.

As used herein, the term "comprising" means "including but not limited to".

As used herein, the term "corn" means *Zea mays* or maize and includes all plant varieties that can be bred with corn, including wild maize species.

As used herein, the term "TC1507 specific" refers to a nucleotide sequence which is suitable for discriminatively identifying event TC1507 in plants, plant material, or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material.

As used herein, the terms "insect resistant" and "impacting insect pests" refers to effecting changes in insect feeding, growth, and/or behavior at any stage of development, including but not limited to: killing the insect; retarding growth; preventing reproductive capability; and the like.

As used herein, the terms "pesticidal activity" and "insecticidal activity" are used synonymously to refer to activity of an organism or a substance (such as, for example, a protein) that can be measured by numerous parameters including, but not limited to, pest mortality, pest weight loss, pest attraction, pest repellency, and other behavioral and physical changes of a pest after feeding on and/or exposure to the organism or substance for an appropriate length of time. For example "pesticidal proteins" are proteins that display pesticidal activity by themselves or in combination with other proteins.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. As used herein, the terms "encoding" or "encoded" when used in the context of a specified nucleic acid mean that the nucleic acid comprises the requisite information to guide translation of the nucleotide sequence into a specified protein. The information by which a protein is encoded is specified by the use of codons. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid or may lack such intervening non-translated sequences (e.g., as in cDNA).

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding

Copy provided by USPTO from the PACR Image Database on 07/26/2004

sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. "Foreign" refers to material not normally found in the location of interest. Thus "foreign DNA" may comprise both recombinant DNA as well as newly introduced, rearranged DNA of the plant. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The site in the plant genome where a recombinant DNA has been inserted may be referred to as the "insertion site" or "target site".

As used herein, "insert DNA" refers to the heterologous DNA within the expression cassettes used to transform the plant material while "flanking DNA" can exist of either genomic DNA naturally present in an organism such as a plant, or foreign (heterologous) DNA introduced via the transformation process which is extraneous to the original insert DNA molecule, e.g. fragments associated with the transformation event. A "flanking region" or "flanking sequence" as used herein refers to a sequence of at least 20 base pair, preferably at least 50 base pair, and up to 5000 base pair which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the original foreign insert DNA molecule. Transformation procedures leading to random integration of the foreign DNA will result in transformants containing different flanking regions characteristic and unique for each transformant. When recombinant DNA is introduced into a plant through traditional crossing, its flanking regions will generally not be changed. Transformants will also contain unique junctions between a piece of heterologous insert DNA and genomic DNA, or 2 pieces of genomic DNA, or 2 pieces of heterologous DNA. A "junction" is a point where 2 specific DNA fragments join. For example, a junction exists where insert DNA joins flanking DNA. A junction point also exists in a transformed organism where 2 DNA fragments join together in a manner that is modified from that found in the native organism. "Junction DNA" refers to DNA that comprises a junction point.

Copy provided by USPTO from the PACR Image Database on 07/20/2004

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous nucleotide sequence can be from a species different from that from which the nucleotide sequence was derived, or, if from the same species, the promoter is not naturally found operably linked to the nucleotide sequence. A heterologous protein may originate from a foreign species, or, if from the same species, is substantially modified from its original form by deliberate human intervention.

"Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements are often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters that cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is

Copy provided by USPTO from the PACR Image Database on 07/26/2004

present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect numerous parameters including, processing of the primary transcript to mRNA, mRNA stability and/or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht *et al.* (1989) *Plant Cell* 1:671-680.

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide.

A DNA construct is an assembly of DNA molecules linked together that provide one or more expression cassettes. The DNA construct may be a plasmid that is enabled for self replication in a bacterial cell and contains various endonuclease enzyme restriction sites that are useful for introducing DNA molecules that provide functional genetic elements, i.e., promoters, introns, leaders, coding sequences, 3' termination regions, among others; or a DNA construct may be a linear assembly of DNA molecules, such as an expression cassette. The expression cassette contained within a DNA construct comprise the necessary genetic elements to provide transcription of a messenger RNA. The expression cassette can be designed to express in prokaryote cells or eukaryotic cells. Expression cassettes of the present invention are designed to express most preferably in plant cells.

The DNA molecules of the invention are provided in expression cassettes for expression in an organism of interest. The cassette will include 5' and 3' regulatory sequences operably linked to a coding sequence of the invention. "Operably linked" means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. Operably linked is intended to indicate a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. The cassette may additionally contain at least one additional gene to be cotransformed into the

Copy provided by USPTO from the PACR Image Database on 07/26/2004

organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes or multiple DNA constructs.

The expression cassette will include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region, a coding region, and a transcriptional and translational termination region functional in the organism serving as a host. The transcriptional initiation region (*i.e.*, the promoter) may be native or analogous, or foreign or heterologous to the host organism. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation.

It is to be understood that as used herein the term "transgenic" includes any cell, cell line, callus, tissue, plant part, or plant the genotype of which has been altered by the presence of a heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

A transgenic "event" is produced by transformation of plant cells with a heterologous DNA construct(s), including a nucleic acid expression cassette that comprises a transgene of interest, the regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and selection of a particular plant characterized by insertion into a particular genome location. An event is characterized phenotypically by the expression of the transgene. At the genetic level, an event is part of the genetic makeup of a plant. The term "event" also refers to progeny produced by a sexual outcross between the transformant and another variety that include the heterologous DNA. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed parent is present in the progeny of the cross at the same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA and flanking sequence immediately adjacent to the inserted DNA that would be expected to be transferred to a progeny that receives inserted DNA including the transgene of interest as the result of a sexual

Copy provided by USPTO from the PACR Image Database on 07/26/2004

cross of one parental line that includes the inserted DNA (e.g., the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA.

An insect resistant TC1507 corn plant can be bred by first sexually crossing a first parental corn plant consisting of a corn plant grown from the transgenic TC1507 corn plant and progeny thereof derived from transformation with the expression cassettes of the present invention that confers insect resistance, and a second parental corn plant that lacks insect resistance, thereby producing a plurality of first progeny plants; and then selecting a first progeny plant that is resistant to insects; and selfing the first progeny plant, thereby producing a plurality of second progeny plants; and then selecting from the second progeny plants an insect resistant plant. These steps can further include the back-crossing of the first insect resistant progeny plant or the second insect resistant progeny plant to the second parental corn plant or a third parental corn plant, thereby producing a corn plant that is resistant to insects.

As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, and progeny of same. Parts of transgenic plants understood to be within the scope of the invention comprise, for example, plant cells, protoplasts, tissues, callus, embryos as well as flowers, stems, fruits, leaves, and roots originating in transgenic plants or their progeny previously transformed with a DNA molecule of the invention and therefore consisting at least in part of transgenic cells, are also an aspect of the present invention.

As used herein, the term "plant cell" includes, without limitation, seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants that can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere *et al.* (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein *et al.* (1987) *Nature* (London) 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Additional transformation methods are disclosed below.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, which are capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels *et al.*, (1985; Supp. 1987) *Cloning Vectors: A Laboratory Manual*, Weissbach and Weissbach (1989) *Methods for Plant Molecular Biology*, (Academic Press, New York); and Flevin *et al.*, (1990) *Plant Molecular Biology Manual*, (Kluwer Academic Publishers). Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several references, e.g., Fehr, in *Breeding Methods for Cultivar Development*, Wilcos J. ed., American Society of Agronomy, Madison Wis. (1987).

A "probe" is an isolated nucleic acid to which is attached a conventional detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, or enzyme. Such a probe is complementary to a strand of a target nucleic acid, in the case of the present invention, to a strand of isolated DNA from corn event TC1507 whether from a corn plant or from a sample that includes DNA from the event. Probes according to the present invention include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that bind specifically to a target DNA sequence and can be used to detect the presence of that target DNA sequence.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

"Primers" are isolated nucleic acids that are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase. Primer pairs of the present invention refer to their use for amplification of a target nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods. "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (see, U.S. Patent Nos. 4,683,195 and 4,800,159; herein incorporated by reference).

Probes and primers are of sufficient nucleotide length to bind to the target DNA sequence specifically in the hybridization conditions or reaction conditions determined by the operator. This length may be of any length that is of sufficient length to be useful in a detection method of choice. Generally, 11 nucleotides or more in length, preferably 18 nucleotides or more, and more preferably 22 nucleotides or more, are used. Such probes and primers hybridize specifically to a target sequence under high stringency hybridization conditions. Preferably, probes and primers according to the present invention have complete DNA sequence similarity of contiguous nucleotides with the target sequence, although probes differing from the target DNA sequence and that retain the ability to hybridize to target DNA sequences may be designed by conventional methods. Probes can be used as primers, but are generally designed to bind to the target DNA or RNA and not be used in an amplification process.

Specific primers can be used to amplify an integration fragment to produce an amplicon that can be used as a "specific probe" for identifying event TC1507 in biological samples. When the probe is hybridized with the nucleic acids of a biological sample under conditions which allow for the binding of the probe to the sample, this binding can be detected and thus allow for an indication of the presence of event TC1507 in the biological sample. Such identification of a bound probe has been described in the art. The specific probe is preferably a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the event and preferably also comprises a part of the foreign DNA contiguous therewith. Preferably the specific probe comprises a sequence of at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, and most preferably between 95 and 100% identical (or complementary) to a specific region of the event.

[redacted]

Copy provided by USPTO from the PACR Image Database on 07/20/2004

Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual, 2nd ed.*, vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*, Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992"); and Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as the PCR primer analysis tool in Vector NTI version 6 (Informax Inc., Bethesda MD); PrimerSelect (DNASTAR Inc., Madison, WI); and Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Additionally, the sequence can be visually scanned and primers manually identified using guidelines known to one of skill in the art.

A "kit" as used herein refers to a set of reagents for the purpose of performing the method of the invention, more particularly, the identification of the event TC1507 in biological samples. The kit of the invention can be used, and its components can be specifically adjusted, for purposes of quality control (e.g. purity of seed lots), detection of event TC1507 in plant material, or material comprising or derived from plant material, such as but not limited to food or feed products. "Plant material" as used herein refers to material which is obtained or derived from a plant.

Primers and probes based on the flanking DNA and insert sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed sequences by conventional methods, e.g., by re-cloning and sequencing such sequences. The nucleic acid probes and primers of the present invention hybridize under stringent conditions to a target DNA sequence. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of DNA from a transgenic event in a sample. Nucleic acid molecules or fragments thereof are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary

to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, 1989, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, a Practical Approach,* IRL Press, Washington, D.C. (1985), departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

In hybridization reactions, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. The thermal melting point (Tm) is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. For DNA-DNA hybrids, the Tm can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: Tm = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. Tm is reduced by about 1°C for each 1% of mismatching; thus, Tm, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the Tm can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the Tm for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the Tm; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the Tm; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the Tm.

Using the equation, hybridization and wash compositions, and desired Tm, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash

Copy provided by USPTO from the PACR Image Database on 07/26/2004

solutions are inherently described. If the desired degree of mismatching results in a Tm of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

As used herein, a substantially homologous sequence is a nucleic acid molecule that will specifically hybridize to the complement of the nucleic acid molecule to which it is being compared under high stringency conditions. Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2X SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of a destabilizing agent such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules unique to the TC1507 event or complements thereof or fragments of either under moderately stringent conditions.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the

Copy provided by USPTO from the PACR Image Database on 07/20/2004

algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0); the ALIGN PLUS program (version 3.0, copyright 1997); and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 10 (available from Accelrys, 9685 Scranton Road, San Diego, CA 92121, USA). Alignments using these programs can be performed using the default parameters.

The CLUSTAL program is well described by Higgins and Sharp, *Gene* 73: 237-244 (1988); Higgins and Sharp, *CABIOS* 5: 151-153 (1989); Corpet, *et al., Nucleic Acids Research* 16: 10881-90 (1988); Huang, *et al., Computer Applications in the Biosciences* 8: 155-65 (1992), and Pearson, *et al., Methods in Molecular Biology* 24: 307-331 (1994). The ALIGN and the ALIGN PLUS programs are based on the algorithm of Myers and Miller (1988) *supra.* The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra.* The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology*, Chapter 19, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). Alignment may also be performed manually by inspection.

To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that

detects distant relationships between molecules. See Altschul *et al.* (1997) supra. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.hlm.nih.gov.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Regarding the amplification of a target nucleic acid sequence (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer

Copy provided by USPTO from the PACR Image Database on 07/26/2004

pair to hybridize only to the target nucleic-acid sequence to which a primer having the corresponding wild-type sequence (or its complement) would bind and preferably to produce a unique amplification product, the amplicon, in a DNA thermal amplification reaction.

The term "specific for (a target sequence)" indicates that a probe or primer hybridizes under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

As used herein, "amplified DNA" or "amplicon" refers to the product of nucleic acid amplification of a target nucleic acid sequence that is part of a nucleic acid template. For example, to determine whether a corn plant resulting from a sexual cross contains transgenic event genomic DNA from the corn plant of the present invention, DNA extracted from the corn plant tissue sample may be subjected to a nucleic acid amplification method using a DNA primer pair that includes a first primer derived from flanking sequence adjacent to the insertion site of inserted heterologous DNA, and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the event DNA. Alternatively, the second primer may be derived from the flanking sequence. The amplicon is of a length and has a sequence that is also diagnostic for the event. The amplicon may range in length from the combined length of the primer pairs plus one nucleotide base pair to any length of amplicon producible by a DNA amplification protocol. Alternatively, primer pairs can be derived from flanking sequence on both sides of the inserted DNA so as to produce an amplicon that includes the entire insert nucleotide sequence of the PHI8999A expression construct, see FIG. 1, approximately 6.2 Kb in size. A member of a primer pair derived from the flanking sequence may be located a distance from the inserted DNA sequence, this distance can range from one nucleotide base pair up to the limits of the amplification reaction, or about twenty thousand nucleotide base pairs. The use of the term "amplicon" specifically excludes primer dimers that may be formed in the DNA thermal amplification reaction.

Nucleic acid amplification can be accomplished by any of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described, inter alia, in U.S. Pat. Nos. 4,683,195 and 4,683,202 and in *PCR Protocols: A Guide to Methods and Applications,* ed. Innis *et al.,* Academic press, San Diego, 1990. PCR amplification methods have been developed to amplify up to 22 Kb of genomic DNA and up to 42 Kb of bacteriophage DNA (Cheng *et al., Proc. Natl. Acad. Sci. USA* 91:5695-5699, 1994). These methods as well as other

Copy provided by USPTO from the PACR Image Database on 07/20/2004

methods known in the art of DNA amplification may be used in the practice of the present invention. It is understood that a number of parameters in a specific PCR protocol may need to be adjusted to specific laboratory conditions and may be slightly modified and yet allow for the collection of similar results. These adjustments will be apparent to a person skilled in the art.

The amplicon produced by these methods may be detected by a plurality of techniques, including, but not limited to, Genetic Bit Analysis (Nikiforov, *et al. Nucleic Acid Res.* 22:4167-4175, 1994) where a DNA oligonucleotide is designed which overlaps both the adjacent flanking DNA sequence and the inserted DNA sequence. The oligonucleotide is immobilized in wells of a microwell plate. Following PCR of the region of interest (using one primer in the inserted sequence and one in the adjacent flanking sequence) a single-stranded PCR product can be hybridized to the immobilized oligonucleotide and serve as a template for a single base extension reaction using a DNA polymerase and labeled ddNTPs specific for the expected next base. Readout may be fluorescent or ELISA-based. A signal indicates presence of the insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Another detection method is the Pyrosequencing technique as described by Winge (*Innov. Pharma. Tech.* 00: 18-24, 2000). In this method an oligonucleotide is designed that overlaps the adjacent DNA and insert DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted sequence and one in the flanking sequence) and incubated in the presence of a DNA polymerase, ATP, sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. dNTPs are added individually and the incorporation results in a light signal which is measured. A light signal indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single or multi-base extension.

Fluorescence Polarization as described by Chen *et al.*, (*Genome Res.* 9:492-498, 1999) is a method that can be used to detect an amplicon of the present invention. Using this method an oligonucleotide is designed which overlaps the flanking and inserted DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted DNA and one in the flanking DNA sequence) and incubated in the presence of a DNA polymerase and a fluorescent-labeled ddNTP. Single base extension results in incorporation of the ddNTP. Incorporation can be measured as a change in polarization using a fluorometer. A change in polarization indicates the presence of the transgene

Copy provided by USPTO from the PACR Image Database on 07/20/2004

insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Taqman® (PE Applied Biosystems, Foster City, Calif.) is described as a method of detecting and quantifying the presence of a DNA sequence and is fully understood in the instructions provided by the manufacturer. Briefly, a FRET oligonucleotide probe is designed which overlaps the flanking and insert DNA junction. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Hybridization of the FRET probe results in cleavage and release of the fluorescent moiety away from the quenching moiety on the FRET probe. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

Molecular Beacons have been described for use in sequence detection as described in Tyangi *et al.* (*Nature Biotech.* 14:303-308, 1996). Briefly, a FRET oligonucleotide probe is designed that overlaps the flanking and insert DNA junction. The unique structure of the FRET probe results in it containing secondary structure that keeps the fluorescent and quenching moieties in close proximity. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Following successful PCR amplification, hybridization of the FRET probe to the target sequence results in the removal of the probe secondary structure and spatial separation of the fluorescent and quenching moieties. A fluorescent signal results. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

A hybridization reaction using a probe specific to a sequence found within the amplicon is yet another method used to detect the amplicon produced by a PCR reaction.

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention, in addition to those shown and described herein, will be apparent to those skilled in the art from

Copy provided by USPTO from the PACR Image Database on 07/20/2004

the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLES

Example 1. Transformation of Maize by Particle Bombardment and Regeneration of Transgenic Plants Containing the Cry1F Gene

A DNA molecule of 6.2 Kb, designated PHI8999A (see FIG. 1 and SEQ ID NO:25), which includes a first transgene expression cassette comprising the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) Plant Mol. Biol. 18:675-689 and Christensen and Quail (1996) Transgenic Res. 5:213-218) operably connected to a DNA molecule encoding a Bacillus thuringiensis δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350), and a second transgene expression cassette comprising a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) operably connected to a DNA molecule encoding the selectable marker, phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene 70*:25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) was used to transform maize embryo tissue.

B.t. Cry1F maize plants were obtained by microprojectile bombardment using the Biolistics® PDS-1000He particle gun manufactured by Bio-Rad, Hercules, CA; essentially as described by Klein *et al.* (1987) *Nature,* UK 327(6117):70-73. Immature embryos isolated from maize ears, harvested soon after pollination were cultured on callus initiation medium for several days. On the day of transformation, microscopic tungsten particles were coated with purified PHI8999A DNA (SEQ ID NO:25) and accelerated into the cultured embryos, where the insert DNA was incorporated into the cell chromosome. Only insert PHI8999A was used during transformation and no additional plasmid DNA was incorporated into the transformant.

After bombardment, embryos were transferred to callus initiation medium containing glufosinate as the selection agent. Individual embryos were kept physically separate during culture, and the majority of explants died on the selective medium.

Those embryos that survived and produced healthy, glufosinate-resistant callus tissue were assigned unique identification codes representing putative transformation events, and continually transferred to fresh selection medium. Plants were regenerated from tissue derived from each unique event and transferred to the greenhouse. Leaf samples were taken for molecular analysis to verify the presence of the transgene by PCR and to confirm expression of the Cry1F protein by ELISA. Plants were then subjected to a whole plant bioassay using European corn borer insects. Positive plants were crossed with inbred lines to obtain seed from the initial transformed plants. A number of lines were evaluated in the field. The TC1507 event was selected from a population of independent transgenic events based on a superior combination of characteristics, including insect resistance and agronomic performance (see Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, fort Collins, CO, herein incorporated by reference).

Example 2. Identification of nucleotides comprising the flanking sequence 5' to the transgenic insert DNA in *Bacillus thuringiensis* Cry1F maize line TC1507

To identify a DNA fragment that included sequence 5' to the PHI8999A insert in event TC1507, Spe I restriction enzyme fragments from event TC1507 genomic DNA were size selected on agarose gels, purified, and screened by Southern analysis to confirm hybridization to a Cry1F probe. Following confirmation of hybridization and fragment size, the fragments of interest were cloned into a pBluescript II SK (+)™ cloning vector to prepare an enriched size selected plasmid based genomic DNA library. A probe homologous to a portion of the Cry1F gene was used to screen the plasmid library for positive clones. A positive clone was identified, purified by additional screening, and confirmed to result in a positive signal when hybridized to the Cry1F probe. Nearly 3 Kb of the Spe I fragment contained in the isolated positive clone was sequenced using a primer walking approach. To initiate the first sequencing run, a primer that binds to a known sequence in the cloning vector DNA was designed to sequence a portion of the DNA of interest. A second sequencing run over the same region using another primer oriented in the reverse direction provided second strand coverage. Primer walking was accomplished by repeatedly using sequence data from previous runs to design new

Copy provided by USPTO from the PACR Image Database on 07/26/2004

primers that were then used to extend the next round of sequencing further into the DNA of interest until the flanking sequence 5' to the inserted transgenic DNA in maize event TC1507 was obtained. Specific sequence information is provided in Example 4.

Example 3. Confirmation of flanking sequence 5' to the *B.t.* Cry1F maize line TC1507 insert

To confirm the 5' flanking sequence of the *B.t.* Cry1F maize line TC1507 insert, PCR primer pairs were designed to obtain overlapping PCR products extending from the 5' flanking region into the full-length PHI8999A transgenic insert. PCR products were successfully amplified from *B.t.* Cry1F maize line TC1507 genomic DNA, isolated, and sequenced for Region 1 through Region 6, shown in Table 1, and confirmed to match the previously determined sequence from the Spe I fragment, described in Example 2. However, the region from bp 2358 to bp 2829, immediately adjacent and 5' to the start of the full-length insert was recalcitrant to PCR amplification and appeared to be larger than the sequence obtained from the Spe I clone described above. The use of primer pairs flanking this region and the Advantage®-GC 2 Polymerase Mix (BD Biosciences Clontech, Palo Alto, CA) was successful in amplifying PCR products from *B.t.* Cry1F maize line TC1507 genomic DNA for sequencing. The amplification conditions used to produce amplicons with the Advantage®-GC 2 system are shown in Table 10. The DNA primer pairs used to confirm the sequence in the region from bp 2358 to 2829 are those listed in SEQ ID NO:1 and SEQ ID NO:2; and SEQ ID NO:2 and SEQ ID NO:23. Sequence from this region is described in Table 1 (Regions 7a, 7b, 7c, and 8).

Example 4. Event TC1507 5' flanking sequence. A description of each region is provided in Table 1.

Region 1 (SEQ ID NO: 28) Maize genomic (no significant homology)

```
   1 ACTAGTTTCC TAGCCCGCGT CGTGCCCCTA CCCCACCGAC GTTTATGGAA
  51 GGTGCCATTC CACGGTTCTT CGTGGCCGCC CCTAAGGATG TAAATGGTCG
 101 GTAAAATCCG GTAAATTTCC GGTACCGTTT ACCAGATTTT TCCAGCCGTT
 151 TTCGGATTTA TCGGGATATA CAGAAAACGA GACGGAAACG GAATAGGTTT
 201 TTTTTCGAAA ACGGTACGGT AAACGGTGAG ACAAACTTAC CGTCCGTTTT
 251 CGTATTTCTC GGGAAACTCT GGTATATTCC CGTATTTGTC CCGTATTTTC
 301 CCGACCCACG GACCTGCCAA TCAACCATCA GCCAGTCAGC CCATCCCCAC
 351 AGCTATGGCC CATGGGGCCA TGTTGGCCAC ATGCCCACGC AACGCAAGGC
 401 AGTAAGGCTG GCAGCCTGGC ACGCATTGAC GCATGTGGAC ACACACAGCC
 451 GCCGCCTGTT CGTGTTTCTG TGCCGTTGTG CGAGACTGTG ACTGCGAGTG
 501 GCGGAGTCGG CGAACGGCGA GGCGTCTCCG GAGTCTGGAC TGCGGCTGTG
```


Copy provided by USPTO from the PACR Image Database on 07/26/2004

551 GACAGCGACG CTGTGACGGC GACTCGGCGA AGCCCCAAGC TACCAAGCCC
601 CCAAGTCCCC ATCCATCTCT GCTTCTCTGG TCATCTCCTT CCCCTGGTCG
651 ATCTGCAGGC GCCAGACCG

Region 2 (SEQ ID NO: 29) Undescribed maize genomic sequence (complement)
670 G CCGAAGCATC ACGAAACGCA CTAAGACCTC
701 GAAGGAGTCA AACCACTCCT CCGAGGCCTC GGGGGCTACA CCCGGCGGGT
751 GCGCTCGCGC GCACCCACCG GAACAAAATG TAACCGAGAA AGGTCGGTCC
801 CCTTGCAAAA AAAGTGCGAC AAAAGCCTCC AAGCGAGTAT TAACACTCAC
851 TTTGAGGCTC GGGGGCTAC

Region 3 (SEQ ID NO: 30) Fragment of maize Huck-1 retrotransposon
870 T GTCGGGGACC ATAATTAGGG GTACCCCCAA
901 GACTCCTAAT CTCAGCTGGT AACCCCCATC AGCACAAAGC TGCAAAGGCC
951 TGATGGGTGC GATTAAGTCA AGGCTCGGTC CACTCAAGGG ACACGATCTC
1001 GCCTCGCCCG AGCCCAGCCT CGGGCAAGGG CGGCCGACCC CGAGGATTCA
1051 CGTCTCGCCC GAGGGCCCCC TCAAGCGACG GGCACACCTT CGGCTCGCCC
1101 GAGGCCCATT CTTCGCCGAG AAGCAACCTT GGCCAGATCG CCACACCGAC
1151 CGACCGTATC GCAGGAGCAT TTAATGCGAG GATCGCCTGA CACCTTATCC
1201 TGACGCGCGC TCTTCAGTCG ACAGAGCCGA AGTGACCGCA ATCACTTCGC
1251 CGCTCCACTG ACCGACCTGA CAAGAAGACA GCGCCGCCTG CGTCGCTCCG
1301 ACTGCTGTGC CACTCGACAG AGTGAGGCTG ACAGCAGCCA AGTCCGGCCT
1351 CGGGCGCCAT AGGAAGCTCC GCCTCGCCCG ACCCTAGGGC TCGGACTCGG
1401 CCTCGGCTCC GGAAGACGAC GAACTACGCT TCGCCCGACC CCAGGGCTTG
1451 GACTCAGCCT CGGCTCCGGA AGACGACGAA TTCCGCCTCG CCCGACCCCA
1501 GGGCTCGGAC TCGGCCTCGG CTCCAGAAGA CGACGAACTC CGCCTCGCCC
1551 GACCCCAGGG CTCGGACTCA GCCTCGGCTC CGGAAGACGA CGAACTCCGC
1601 CTCGCCCGAC CCCAGGGCTC GGACTCAGCC TCGGCCTCAG ACGATGGTCT
1651 CCGCCTCGCC CGACCCGGGG CTCGGACTCG A

Region 4 (SEQ ID NO: 31) Fragment of *cry*1F gene
1682 CCTTTCTAT CGGACCTTGT
1701 CAGATCCTGT CTTCGTCCGA GGAGGCTTTG GCAATCCTCA CTATGTACTC
1751 GGTCTTAGGG GAGTGGCCTT TCAACAAACT GGTACGAATC ACACCCGCAC
1801 ATTCAGGAAC TCCGGGACCA TTGACTCTCT AGATGAGATA CCACCTCAAG
1851 ACAACAGCGG CGCACCTTGG AATGACTACT CCCATGTGCT GAATCATGTT
1901 ACCTTTGTGC GCTGGCCAGG TGAGATCTCA GGTTCCGACT CATGGAGAGC
1951 ACCAATGTTC TCTTGGACGC ATCGTAGCGC TACCCCCACA AACACCATTG
2001 ATCCAGAGAG AATCAC

Region 5 (SEQ ID NO: 32) Fragment of maize chloroplast *rpo*C2 gene
2017 TCAT TCTTCAAGAA CTGCATATCT TGCCGAGATC
2051 CTCATCCCTA AAGGTACTTG ACAATAGTAT TATTGGAGTC GATACACAAC
2101 TCACAAAAAA TACAAGAAGT CGACTAGGTG GATTGGTCCG
AGTGAAGAGA
2151 AAAAAAAGCC ATACAGAACT CAAAATCTTT TCCGGAGATA TTCATTTTCC

Copy provided by USPTO from the PACR Image Database on 07/20/2004

2201 TGAAGAGGCG GATAAGATAT TAGGTGGCAG TTTGATACCA
CCAGAAAGAG
2251 AAAAAAAAGA TTCTAAGGAA TCAAAAAAAA GGAAAAATTG
GGTTTATGTT
2301 CAACGGAAAA AATTTCTCAA AAGCAAGGAA AAGTATT

Region 6 (SEQ ID NO: 33) Fragment of maize chloroplast or *ubi*ZM1(2) promoter
2338 GTG GCTATTTATC
2351 TATC

Nucleotides 2355-2358 (CGT) connect Region 6 to Region 7a.

Region 7a (SEQ ID NO: 34) Fragment of *pat* gene
2358 GCA GCTGATATGG CCGCGGTTTG TGATATCGTT AACCATTACA
2401 TTGAGACGTC TACAGTGAAC TTTAGGACAG AGCCACAAAC ACCACAAGAG
2451 TGGATTGATG ATCTAGAGAG GTTGCAAGAT AGATACCCTT GGTTGGTTGC
2501 TGAGGTTGAG GGTGTTGTGG CTGGTATTGC TTACGCTGGG CCCTGGAAGG
2551 CTAGGAAC

Region 7b (SEQ ID NO: 35) Fragment of *pat* gene (complement)
2559 CC TCAACCTCAG CAACCAACCA ATGGTATCTA TCTTGCAACC
2601 TCTCTAGATC ATCAATCCAC TCTTGTGGTG TTTGTGGCTC TGTCCTAAAG
2651 TTCACTGTAG ACGTCTCAAT GTAATGGTTA ACGATATCAC AAACCG

Region 7c (SEQ ID NO: 36) Fragment of *cry*1F gene (complement)
2697 AGAG
2701 AAGAGGGATC T

Region 8 (SEQ ID NO: 37) Fragment of Polylinker
2712 CGAAGCTTC GGCCGGGGCC CATCGATATC CGCGGGCATG
2751 CCTGCAGTGC AGCGTGACCC GGTCGTGCCC CTCTCTAGAG ATAATGAGCA
2801 TTGCATGTCT AAGTTATAAA AAATTACCA

Region 9 (SEQ ID NO: 25) Full-length insert of PHI8999A

Example 5. Description of the flanking sequence 5' to the insert in maize event TC1507

In order to more fully describe the event TC1507 5' flanking sequence, homology searching was done against the GenBank public databases (release 122, 2/01) using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, pairwise alignments were performed using AlignX (InforMax Inc., Bethesda, MD) to look for homology between the maize event TC1507

Copy provided by USPTO from the PACR Image Database on 07/20/2004

flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The TC1507 5' flanking sequence is numbered with base 1 being the furthest 5' to the insert and base 2830 at the starting point of the full-length PHI8999A transgenic insert (see FIG. 1). The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed.

In most cases, similarity searching with the event TC1507 5' flanking sequence resulted in a match to one unique sequence based on a very high percent identity value. Those sequences are identified in Table 1. In addition, there are two regions in the TC1507 5' DNA flanking sequence with high similarity to more than one known sequence. In regions 870-1681 and 2338-2354, the percent identity scores with both sequence fragments are sufficiently high that a single match (homolog) cannot be determined. The two possible homologs for each of these regions are indicated in Table 1.

Highly similar sequences were identified for all but the first 669 base pairs of sequence. Generally, the results of similarity searching indicate high homology with maize genomic sequences 5' to base 1681. The region from base 1682 to the start of the PHI8999A insert at position 2830 contains some fragments associated with the transformation event.

Table 1. Sequence summary for event TC1507 insert

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
1	1-669	669	N/A[1]	N/A	N/A	No significant homology detected
2	670-869	200	90.5	AF123535	52432-52632 (complement)	Undescribed maize genomic sequence
3	870-1681	812	89.4	AF050439	1-801	Fragment of maize Huck-1 retrotransposon 5' LTR[2]
			86.6	AF050438	1-797	Fragment of maize Huck-1 retrotransposon 3' LTR

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
4	1682-2016	335	100.0	PHI8999A	3149-3483	Fragment of *cry*1F gene
5	2017-2337	321	100.0	X86563	29429-29749	Fragment of maize chloroplast *rpoC2* gene (RNA polymerase beta-2 subunit)
6	2338-2354	17	100.0	X86563	97643-97659	Fragment of maize chloroplast *trnI* gene (tRNA-Ile)
			82.4	PHI8999A	182-197	Fragment of maize *ubi*ZM1(2) promoter
7a	2358-2558	201	100.0	PHI8999A	5320-5475	Fragment of *pat* gene
7b	2559-2696	138	99	PHI8999A	5336-5518 (complement)	Fragment of *pat* gene
7c	2697-2711	15	100.0	PHI8999A	2544-2558 (complement)	Fragment of *cry*1F gene
8	2712-2829	118	100.0	PHI8999A	36-153	Fragment of polylinker region (bases 36-80) and *ubi*ZM1(2) promoter (bases 81-153)
9	2830-9015	6186	100.0	PHI8999A	11 – 6196	Full-length insert of PHI8999A
10	9016-9565	550	100.0	PHI8999A	3906-4456 (complement)	Inverted ORF25 terminator
11	9566-9693	128	100.0	NC_001666	121851-121978 (complement) & 100759-100886	Fragment of maize chloroplast *rps12* rRNA (23S ribosomal RNA)
12	9696-10087	392	99	NC_001666	17091-17483 (complement)	Fragment of maize chloroplast genome
13	10088-10275	188	99	PHI8999A	5333-5520 (complement)	Fragment of *pat* gene

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
14	10278-10358	81	100	NC_001666	137122-137202 (complement)	Fragment of maize chloroplast "ORF241" – hypothetical protein gene
15	10359-10612	254	N/A[1]	N/A	N/A	No significant homology detected
16	10613-11361	749	N/A[1]	N/A	N/A	No description available

[1] N/A; not applicable
[2] LTR; long terminal repeat

Example 6: Confirmation of the presence of Regions 1, 2, and 3 in an unmodified control corn line

PCR analysis was used to determine if Regions 1, 2, and 3 (Table 1) in the 5' flanking region of Event TC1507 are present in an unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Nine different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified control corn line Hi-II (see Armstrong (1994) *The Maize Handbook*, ed. Freeling and Walbot, Springer-Verlag, New York, pp. 663-671, for information on Hi-II) as outlined in Table 2 using the primer sequences shown in Table 3. Two reactions were designed to amplify DNA within Region 1 of the 5' flanking region from bp 25 to 324 (Reaction A - 300 bp amplicon); and from bp 25 to 480 (Reaction B - 456 bp amplicon). The expected amplicons were present in both the Hi-II unmodified corn line and in maize event TC1507. One PCR primer pair, Reaction C, spanned Region 2 to Region 3 of the 5' flanking region from bp 759 to 1182 (424 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reaction D, spanned Region 1 to Region 3 of the 5' flanking region from bp 415 to 1182 (768 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reactions E and F were designed as specific primer pairs for the *pat* gene region of the full-length insert of PHI8999A in TC1507 and thus an amplicon in the unmodified Hi-II corn line is not expected. The results indicate that both Reactions E and F are specific for a

Copy provided by USPTO from the PACR Image Database on 07/26/2004

maize line transformed with a *pat* gene region and produce the expected amplicon, whereas no amplicon was produced in the unmodified Hi-II corn line. Reaction G was also designed as a primer pair that would produce an amplicon of 366 bp in the maize event TC1507 and no amplicon in the unmodified Hi-II corn line.

Reactions H and I were designed as specific primer pairs for TC1507 that would span the end of the transgenic insert into the 5' flanking region. In both Reactions H and I, the reverse primer was located in the ubiquitin promoter region of the full-length PHI8999A insert (Region 9 in Table 1) and the forward primer was located in Region 5, the *rpoC2* gene fragment (see Table 1). Reaction H and Reaction I both produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. These results indicate that both Reactions H and I are specific for the TC1507 event.

The PCR results show that the undescribed sequence (Region 1) is present in the unmodified corn line Hi-II and that Regions 1, 2 and 3, are contiguous in the unmodified corn line Hi-II. The DNA sequences amplified in Reactions A, B, C, and D are not unique to the 5' flanking region of maize event TC1507 but are also present in the unmodified corn line Hi-II.

Table 2. PCR reactions for sequence 5' to the PHI8999A insert in maize event TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
A	25 – 324 bp in TC1507 flanking sequence	300	Region 1	Yes	Yes
B	25 – 480 bp in TC1507 flanking sequence	456	Region 1	Yes	Yes
C	759 – 1182 bp in TC1507 flanking sequence	424	Region 2 to Region 3	Yes	Yes

Copy provided by USPTO from the PAIR Image Database on 07/26/2004

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
D	415 – 1182 bp in TC1507 5' flanking sequence	768	Region 1 to Region3	Yes	Yes
E Not Unique to TC1507	4750 – 5794 bp in PHI8999A	1045	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes
F Not Unique to TC1507	4827 - 5308 bp in PHI8999A	482	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes
G Detects cry1F fragment in 5' flanking region	*cry*1F sequence in 5' flanking sequence and in full-length insert of PHI8999A	366	Spans 335 bp *cry*1F sequence in 5' flanking sequence and same sequence in the full-length insert	No	Yes
H Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3069 bp in Region 9 (full-length insert of PHI8999A)	912	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes
I Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3001 bp in Region 9 (full-length insert of PHI8999A)	844	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Table 3. PCR primers for sequence 5' to the PHI8999A insert in TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
A	300	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:11	TTGATTGGCAGGTCCGTGGGTC
B	456	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:12	CACAACGGCACAGAAACACGAA
C	424	SEQ ID NO:13	GCGCACCCACCGGAACAAAATG
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
D	768	SEQ ID NO:15	CCTGGCACGCATTGACGCATGT
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
E	1045	SEQ ID NO:6	TAGAGGACCTAACAGAACTCGCCGT
		SEQ ID NO:7	GAGCTGGCAACTCAAAATCCCTTT
F	482	SEQ ID NO:8	AAAATCTTCGTCAACATGGTGGAGC
		SEQ ID NO:9	TAATCTCAACTGGTCTCCTCTCCGG
G	366	SEQ ID NO:19	GGCTCGGACTCGACCTTTCTAT
		SEQ ID NO:20	GCAGTTCTTGAAGAATGAGTGA
H	912	SEQ ID NO:1	GTAGTACTATAGATTATATTATTCGT AGAG
		SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
I	844	SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
		SEQ ID NO:23	CTTCAAACAAGTGTGACAAA

Example 7. Flanking sequence 3' to inserted transgenic DNA in maize event TC1507

Two separate PCR approaches were used to extend the length of the sequence information 3' to the full-length PHI8999A insert in maize event TC1507. In the first approach PCR primer pairs were designed to amplify a product that spanned the junction between the full-length insert and the inverted ORF25 terminator, see FIG. 1 for a depiction of the inverted ORF25 terminator. A forward primer was located at the end of the full-length PHI8999A insert and a series of reverse primers were located at 100 bp intervals in the inverted sequence. In this manner the length of the inverted fragment present in the maize event TC1507 could be determined within a 100 bp region based on the successful PCR reactions. This method

Copy provided by USPTO from the PAIR Image Database on 07/20/2004

indicated the inverted fragment contained the majority of the ORF25 terminator but no Cry1F sequence. PCR fragments were isolated and sequenced from this region.

In the second approach PCR primers were designed to walk out into the flanking DNA sequence from the inverted ORF25 terminator region as determined in the PCR experiment described above. Genomic DNA isolated from two to three individual plants of event TC1507 and an unmodified control corn line was digested with various restriction enzymes and then ligated to adaptors specific for the restriction enzyme used for digestion (Universal Genome Walker™ Kit, Clontech Laboratories, Inc. and Devon *et al.* (1995) *Nucleic Acids Res.* 23:1644-1645). Primary PCR was carried out using an ORF25 terminator specific primer and a primer homologous to the adaptor sequence ligated onto the digested DNA. In order to increase the specificity of the reaction a nested secondary PCR was performed again with another ORF25 terminator specific primer and a secondary primer homologous to the adaptor sequence with the secondary primers being internal to the respective primers used in the primary PCR. Products produced by the nested PCR were analyzed by agarose gel electrophoresis and fragments unique to TC1507 DNA samples were isolated and sequenced. Fragments were amplified from both the ORF25 terminator contained within the full-length insert and from the targeted (inverted) ORF25 terminator on the 3' end of the full-length PHI8999A insert. Fragments from the full-length insert were of a predicted size based on the knowledge of the restriction enzyme sites located in the full-length insert. Fragments produced from the 3' inverted ORF25 terminator appeared as fragments of unexpected size. Sequence analysis of amplified fragments from the 3' inverted ORF25 terminator resulted in flanking DNA sequence of 1043 bp. Resultant sequence from the above series of genome walking experiments was used to design additional primers to walk further out from the insert into the bordering maize genome with a final 3' flanking sequence, of 2346 bp.

In order to describe the TC1507 3' flanking sequence, homology searching was done against the GenBank public databases using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, alignments were performed using SeqMan 4.05™, Martinez and Needleman-Wunsch alignment algorithms (DNASTAR Inc.) to look for homology between the TC1507 3' flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The percent identity values indicate the percentage of identical matches

Copy provided by USPTO from the PACR Image Database on 07/26/2004

across the length of the sequences analyzed. The results of similarity searching for the 3' flanking sequence indicate high homology with three regions of maize chloroplast DNA, a 188 bp fragment of the *pat* gene, and 254 bp of DNA (Region 15, Table 1) with no significant homology. An additional 749 bp (Region 16) beyond Region 15 (see Table 1) was also sequenced. No similarity searching results are available for Region 16.

PCR analysis on control and TC1507 genomic DNA determined that the 254 bp sequence (Region 15, fragment of maize chloroplast "ORF241") is present in the maize genome. The DNA sequence of Region 15 in the 3' flanking region is not unique to the 3' flanking region of maize event TC1507 but is also present in the unmodified control corn line. The TC1507 3' flanking sequence is presented in Example 8 and diagrammed in FIG. 1.

Example 8. Sequence of the region 3' to the end of the full-length insert DNA in maize event TC1507. A description of each region is in Table 1.

Region 10 (SEQ ID NO: 38) Fragment of ORF25 Terminator (complement)

9016 CTCAC TCCGCTTGAT CTTGGCAAAG ATATTTGACG
9051 CATTTATTAG TATGTGTTAA TTTTCATTTG CAGTGCAGTA TTTTCTATTC
9101 GATCTTTATG TAATTCGTTA CAATTAATAA ATATTCAAAT CAGATTATTG
9151 ACTGTCATTT GTATCAAATC GTGTTTAATG GATATTTTTA TTATAATATT
9201 GATGATATCT CAATCAAAAC GTAGATAATA ATAATATTTA TTTAATATTT
9251 TTGCGTCGCA CAGTGAAAAT CTATATGAGA TTACAAAATA CCGACAACAT
9301 TATTTAAGAA ACATAGACAT TAACCCTGAG ACTGTTGGAC ATCAACGGGT
9351 AGATTCCTTC ATGCATAGCA CCTCATTCTT GGGGACAAAA GCACGGTTTG
9401 GCCGTTCCAT TGCTGCACGA ACGAGCTTTG CTATATCCTC GGGTTGGATC
9451 ATCTCATCAG GTCCAATCAA ATTTGTCCAA GAACTCATGT TAGTCGCAAC
9501 GAAACCGGGG CATATGTCGG GTATCTCGAG CTCGCGAAAG CTTGGCTGCA
9551 GGTCGACGGA TCCTT

Region 11 (SEQ ID NO: 39) Fragment of maize chloroplast *rps12* rRNA gene (complement)

9566 CAACA AAAGGGTACC TGTACCCGAA ACCGACACAG
9601 GTGGGTAGGT AGAGAATACC TAGGGGCGCG AGACAACTCT CTCTAAGGAA
9651 CTCGGCAAAA TAGCCCCGTA ACTTCGGGAG AAGGGGTGCC CCC

Nucleotides 9694-9695 (CG) connect Region 11 to Region 12.

Region 12 (SEQ ID NO: 40) Fragment of maize chloroplast genome

9696 CTAAC
9701 AATAAACGAA TACGGTTTAT GTATGGATTC CGGTAAAATA CCGGTACTCG
9751 ATTTCATAAG AGTCGAATAG GAAGTTAAGA TGAGGGTGGT ATCATCATAA
9801 AAATGGAGTA GTATCCTAAA TTATACTAAT CCACGTATGA TATGTATGCC
9851 TTTCCTTATC AACCGGAAGT AGTGCAAAAA AAATTCTATA CTGCACTGCT

Copy provided by USPTO from the PACR Image Database on 07/26/2004

9901 CTCTTTTTAC TGAGAAATGC AAAAAAATAA AAGTGAAGTA AGGGTGCCCC
9951 ATAGATATTT GATCTTGCCT CCTGTCCCCC CCCCCCTTTT TTCATCAAAA
10001 ATTTCCATGA AAAAAGAAAA GATGAATTTG TCCATTCATT GAACCCTAGT
10051 TCGGGACTGA CGGGGCTCGA ACCCGCAGCT TCCGCCT

Region 13 (SEQ ID NO: 41) **Fragment of *pat* gene (complement)**
10088 GTT CCTAGCCTTC
10101 CAGGGCCCAG CGTAAGCAAT ACCAGCCACA GCACCCTCAA
CCTCAGCAAC
10151 CAACCAAGGG TATCTATCTT GCAACCTCTC TAGATCATCA ATCCACTCTT
10201 GTGGTGTTTG TGGCTCTGTC CTAAAGTTCA CTGTAGACGT CTCAATGTAA
10251 TGGTTAACGA TATCACAAAC CGCGG

Nucleotides 10276-10277 (AA) connect Region 13 to Region 14.

Region 14 (SEQ ID NO: 42) Fragment of maize chloroplast ORF241 (complement)
10278 CAC AAGAACGAAA GCACCTTTTC
10301 ATTCTTTCAT ATACTAGGGG TTTTTACTTG GAAAAGACAA TGTTCCATAC
10351 TAAAGGAT

Region 15 (SEQ ID NO: 43) **Maize genomic (no significant homology)**
10359 AG CTGCAGAAGC CGCCACCGTC TTGAGGACCT TCCGGGGAGC
10401 CAGACCGGTC GAACCGTGCC TCCACTTGCT AAGGAGAAAG
GGAAAATCAG
10451 GGCCAGGACA TACGAAGGAG GAGCCAGAAC GAAGATATCC
TAAGATACTT
10501 ACTCGCTCCG GGCCATGATC AATCATGCCT GTGGGGAGGT CTCTCGCACC
10551 TCGATCCATG AAGGTACCAC CGAGGTCTGC CCCGCCGCCG GCTTCGGTAC
10601 CGTCCTCGCC TT

Region 16 (SEQ ID NO: 44) Maize genomic
10613 GGGCGCCC GAGGCACCCG GGGGATGGAC TGCCCAGGCG
10651 CAGCCACGAC GACCCAAGGA TCACCCTCCT GCGCAGTCGG
CACGAGCAAT
10701 AGTTCTCGGG GAACAGGCAG CTTGGCCTGA CTCCCCGGGG TCACCTCAAC
10751 TACCTCGGCC GAGGGGTCAA GTACCCCCTC AGTCCGCCCC CGCTCTTCGG
10801 ACCGGGACCC CGACGTCCCG GCCCCGGATA CCGACGGCAC CAGCCCGCTC
10851 GGGGGCTGGC TTGACGACCC CTGGCCCAGC CTCAGATCTG GGCTGAGGCC
10901 GAGGCAGGCG GCCATGTCGT CGTCTTCATC ATCGTCTTCA TCATCGTCGT
10951 CGTCATCAGG CGTCTCCGGC GACGGCTCCC TTGGGAGCCC CTCCCTCTCC
11001 TGCCGACGAC GAAGCCTTTC CAAGGCATCC CGAGCCCACG TCCGCTCGTG
11051 GGCCCGAGCC TTCTTTGCGT CCTTCTTCTC CTTCCTCTTC TCCGCGGTGA
11101 CCCTCCGCGC AGCTCGGTCC ACCGCATCCT CCGGGACTGG TGGCAGGGAA
11151 GGCTTGTGAT GCCCTACCTC CTGGAGACAG ACGAAAAGTC TCAGCTATGA
11201 GAACCGAGGG CAATCTGACG CAAGAAGGAA GAAGGAGCGG
ATACTCACCA

Copy provided by USPTO from the PACR Image Database on 07/20/2004

11251 GAGACACGCA CCCGCGATCG GGACGCATTA AGGGCTGGGA
AAAAGTGCCG
11301 GCCTCTAATT TCGCTACCGT GCCGTCCACC CACCTGTGGA GGTCATCGAT
11351 GGGAAGGGGA A

Example 9. Confirmation of the presence of Region 15 in the unmodified control corn line

PCR analysis was used to determine if the undescribed region of sequence on the end of the 3' flanking sequence (Region 15 in Table 1) is present in the unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Successful PCR amplification of Region 15 in both maize line TC1507 and the unmodified Hi-II control corn line revealed that Region 15 was indeed present in corn genomic DNA. Five different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified Hi-II control corn line as outlined in Table 7 below using the primer sequences shown in Table 8. Three reactions were designed to amplify DNA within Region 15 of the 3' flanking region; Reaction L – producing a 175 bp amplicon, Reaction M – producing a 134 bp amplicon, and Reaction N – producing a 107 bp amplicon. The expected amplicons were present in both the unmodified control corn line and in maize line TC1507. Reactions J and K were designed as specific primer pairs for TC1507 that would span the end of the insert into the 3' flanking region. In Reaction J, the forward primer was located in the *pat* gene fragment on the 3' end of the full-length PHI8999A insert (Region 13 in Table 1) and the reverse primer was located in the undefined Region 15. In Reaction K the forward primer was located in the chloroplast hypothetical protein gene on the 3' end of the full-length insert (Region 14 in Table 1) and the reverse primer was located in the undefined Region 15. Both Reaction J and Reaction K produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. The results indicate that both Reactions J and K are specific for the TC1507 event.

The PCR results indicate that the undescribed sequence (Region 15) of the 3' flanking sequence of TC1507 is also present in genomic DNA isolated from the unmodified Hi-II control corn line. The DNA sequences amplified in Reactions L, M, and N are not unique to the 3' flanking region of TC1507 but are also present in the unmodified control corn line.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Table 7. PCR reactions for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Region in TC1507 3' flanking sequence	Amplicon present in Control	Amplicon present in maize line TC1507
J	342	Region 13 (*pat* gene fragment) to Region 15	No	Yes
K	252	Region 14 (chloroplast gene) to Region 15	No	Yes
L	175	Region 15	Yes	Yes
M	134	Region 15	Yes	Yes
N	107	Region 15	Yes	Yes

Table 8. PCR primers for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
J	342	SEQ ID NO:3	TGTGGTGTTTGTGGCTCTGTCCTAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
K	252	SEQ ID NO:4	AGCACCTTTTCATTCTTTCATATAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
L	175	SEQ ID NO:16	AAGCCGCCACCGTCTTGAGGACCTT
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
M	134	SEQ ID NO:17	GTCGAACCGTGCCTCCACTTGCTAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
N	107	SEQ ID NO:18	AGAAAGGGAAAATCAGGGCCAGGAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC

Copy provided by USPTO from the PACR Image Database on 07/20/2004

Example 10. PCR Primers

DNA event specific primer pairs were used to produce an amplicon diagnostic for TC1507. These event primer pairs include, but are not limited to, SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 2 and SEQ ID NO: 23; SEQ ID NO: 3 and SEQ ID NO: 5; and SEQ ID NO: 4 and SEQ ID NO: 5. In addition to these primer pairs, any primer pair derived from SEQ ID NO: 26 and SEQ ID NO: 27 that when used in a DNA amplification reaction produces a DNA amplicon diagnostic for TC1507 is an aspect of the present invention. The amplification conditions for this analysis are illustrated in Table 9, however, any modification of these methods that use DNA primers or complements thereof to produce an amplicon DNA molecule diagnostic for TC1507 is within the ordinary skill of the art. The preferred amplification conditions for reactions utilizing the PCR primers identified in SEQ ID NOS: 1, 2, and 23 are illustrated in Table 10. In addition, control primer pairs, which include SEQ ID NOS: 10 and 11; SEQ ID NOS: 10 and 12; SEQ ID NOS: 13 and 14; SEQ ID NOS: 14 and 15; SEQ ID NOS: 5 and 16; SEQ ID NOS: 5 and 17; and SEQ ID NOS: 5 and 18; for amplification of an endogenous corn gene are included as internal standards for the reaction conditions. Also included are primer pairs that will produce an amplicon in transgenic events containing a *pat* gene (SEQ ID NOS: 6 and 7; SEQ ID NOS: 8 and 9), and a primer pair that will produce an amplicon in transgenic events containing a *cry*1F gene (SEQ ID NOS: 19 and 20).

The analysis of plant tissue DNA extracts to test for the presence of the TC1507 event should include a positive tissue DNA extract control (a DNA sample known to contain the transgenic sequences). A successful amplification of the positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences. A negative, or wild-type, DNA extract control in which the template DNA provided is either genomic DNA prepared from a non-transgenic plant, or is a non-TC1507 transgenic plant, should also be included. Additionally a negative control that contains no template corn DNA extract will be a useful gauge of the reagents and conditions used in the PCR protocol.

Additional DNA primer molecules of sufficient length can be selected from SEQ ID NO: 26 and SEQ ID NO: 27 by those skilled in the art of DNA amplification methods, and conditions optimized for the production of an amplicon that may differ from the methods shown in Table 9 or Table 10 but result in an amplicon diagnostic for event TC1507. The use of these DNA primer sequences with modifications to the methods shown in Table 9 and Table 10 are within the scope of the invention. The amplicon wherein at least one DNA primer

Copy provided by USPTO from the PACR Image Database on 07/26/2004

molecule of sufficient length derived from SEQ ID NO: 26 and SEQ ID NO: 27 that is diagnostic for event TC1507 is an aspect of the invention. The amplicon wherein at least one DNA primer of sufficient length derived from any of the genetic elements of PHI8999A that is diagnostic for event TC1507 is an aspect of the invention. The assay for the TC1507 amplicon can be performed by using a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler, or by methods and apparatus known to those skilled in the art.

Table 9. PCR Conditions:

Conditions:

Kit used:	Perkin-Elmer AmpliTAQ Gold kit
Volume	Component
5 µl	template (10 ng/µl)
4 µl	2 µl each primer (10 µM)
2 µl	10X PCR Gold Buffer
2 µl	25 mM $MgCl_2$
2 µl	50X dNTP's (10 mM)
0.1 µl	Amplitaq Gold Polymerase
4.9 µl	H_2O
20 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

9 min 92°C
30 cycles:
94°C 30 sec
60°C 30 sec
72°C 1 min
7 min 72°C
Hold 4°C

Copy provided by USPTO from the PACR Image Database on 07/20/2004

Table 10. PCR Conditions used with the Advantage®-GC 2 Polymerase Mix:

Conditions:

Kit used:	Advantage®-GC 2 Polymerase Mix
Volume	Component
5 µl	template (10 ng/µl)
5 µl	2.5 µl each primer (10 µM)
10 µl	5x GC2 Buffer
10 µl	GC melt (1.0 M final conc.)
1.5 µl	50X dNTP's (10 mM)
1.0 µl	Advantage GC2 Polymerase
17.5 µl	H_2O
50 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

5 min 94°C
35 cycles:
94°C 1 min
60°C 2 min
72°C 3 min
7 min 72°C
Hold 4°C

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications that are within the spirit and scope of the appended claims.

All publications and published patent documents cited in this specification are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

WHAT IS CLAIMED IS:

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21.
2. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:22.
3. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:24.
4. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:26.
5. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:27.
6. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising at least a first primer, which recognizes a sequence within SEQ ID NO:21 or within SEQ ID NO:22.
7. The kit of claim 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
8. The kit of claim 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
9. The kit of claim 6, wherein the first primer recognizes a sequence within SEQ ID NO:21.
10. The kit of claim 6, wherein the first primer recognizes a sequence within SEQ ID NO:22.
11. The kit of claim 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.
12. The kit of claim 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.
13. The kit of claim 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.
14. The kit of claim 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.
15. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA

Copy provided by USPTO from the PACR Image Database on 07/20/2004

polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 26.

16. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 27.

17. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with SEQ ID NO:21 and SEQ ID NO:25, contiguous therewith.

18. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

19. A DNA detection kit comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny.

20. A DNA construct comprising: a first and second expression cassette, wherein said first expression cassette in operable linkage comprises

    (i) a maize ubiquitin promoter;

    (ii) a 5' untranslated exon of a maize ubiquitin gene;

    (iii) a maize ubiquitin intron;

    (iv) a Cry1F encoding DNA molecule; and

    (v) a 3' ORF25 transcriptional terminator; and

said second expression cassette comprising in operable linkage

    (a) a CaMV 35S promoter;

    (b) a *pat* encoding DNA molecule; and

    (c) a 3' transcriptional terminator from (CaMV) 35S.

21. A plant comprising the DNA construct of claim 20.

22. A plant of claim 21, wherein said plant is a corn plant.

23. A method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22.

Copy provided by USPTO from the PACR Image Database on 07/20/2004

24. The method of claim 23, further comprising amplifying a DNA fragment from a nucleic acid present in said biological sample using a polymerase chain reaction with at least two primers, wherein said first primer recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, and a second primer recognizes a sequence within SEQ ID NO:22 or SEQ ID NO: 25.
25. The method of claim 24, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.
26. The method of claim 23, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.
27. The method of claim 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively.
28. The method of claim 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.
29. The method of claim 26, wherein said first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively.
30. The method of claim 26, wherein said first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively.
31. The method of claim 27, comprising amplifying a fragment of about 912 bp using a TC1507 PCR identification protocol.
32. The method of claim 28, comprising amplifying a fragment of about 844 bp using a TC1507 PCR identification protocol.
33. The method of claim 29, comprising amplifying a fragment of about 342 bp using a TC1507 PCR identification protocol.
34. The method of claim 30, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol.
35. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:
    (a) extracting a DNA sample from said biological sample;
    (b) providing DNA primer molecules SEQ ID NO:1 and SEQ ID NO:2 ;
    (c) providing DNA amplification reaction conditions;
    (d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

Copy provided by USPTO from the PACR Image Database on 07/26/2004

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

36. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:2 and SEQ ID NO:23 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

37. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:3 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

38. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:4 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

Copy provided by USPTO from the PACR Image Database on 07/26/2004

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

39. An isolated DNA molecule comprising the amplicon produced by the method of claim 35.

40. An isolated DNA molecule comprising the amplicon produced by the method of claim 36.

41. An isolated DNA molecule comprising the amplicon produced by the method of claim 37.

42. An isolated DNA molecule comprising the amplicon produced by the method of claim 38.

43. A method of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:

a. contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;

b. subjecting the sample and probe to stringent hybridization conditions; and

c. detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event.

44. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 or its complement.

45. An isolated DNA nucleotide primer sequence of claim 44 comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement.

46. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:26 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

47. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.

48. An isolated DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

49. A method for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample.

50. A method for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot.

51. An insect resistant corn plant, or parts thereof, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

52. A descent plant of the insect resistant corn plant of claim 51 wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

53. Seed of a plant of claim 51 or 52.

54. A method of producing an insect resistant corn plant comprising breeding with a plant of claim 51 or 52, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

55. An isolated DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

56. A pair of isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce an amplicon diagnostic for event TC1507.

57. The pair of isolated DNA sequences of claim 56 wherein each is chosen from SEQ ID NO:26.

58. The pair of isolated DNA sequences of claim 56 wherein each is chosen from SEQ ID NO:27.

59. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is on opposite sides of a sequence diagnostic for said TC1507 event insertion;

(b) contacting a sample of said corn tissue with said primer pair;

(c) performing DNA amplification and analyzing for an amplicons.

60. The method of claim 59 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

61. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) subjecting said sample and probe to stringent hybridization conditions; and

(c) analyzing for hybridization of the probe.

62. A DNA detection kit comprising a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

Copy provided by USPTO from the PACR Image Database on 07/20/2004

63. A DNA detection kit comprising a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion.

64. The DNA detection kit of claim 61 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

65. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

66. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

Copy provided by USPTO from the PACR Image Database on 07/20/2004

ABSTRACT OF THE DISCLOSURE

The invention provides DNA compositions that relate to transgenic insect resistant maize plants. Also provided are assays for detecting the presence of the maize TC1507 event based on the DNA sequence of the recombinant construct inserted into the maize genome and the DNA sequences flanking the insertion site. Kits and conditions useful in conducting the assays are provided.

Copy provided by USPTO from the PACR Image Database on 07/26/2004

Copy provided by USPTO from the PAIR image database on 07/20/2004

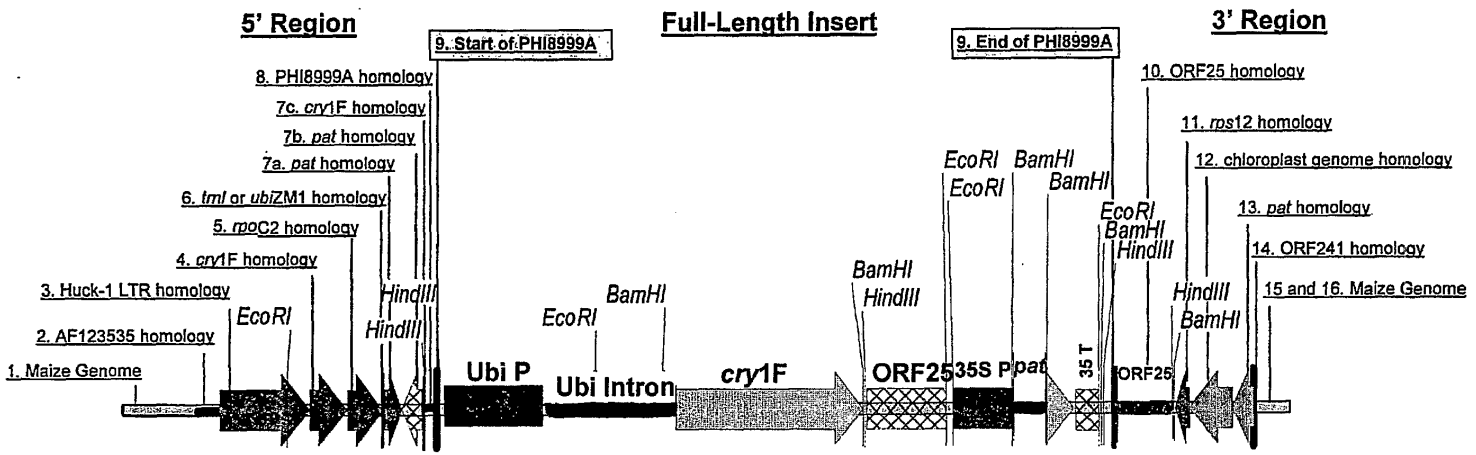


FIG. 1

SEQUENCE LISTING

<110> Mary E. Hartnett Locke

<120> Corn Event TC1507 and Methods for
Detection Thereof

<130> 1523P

<160> 57

<170> FastSEQ for Windows Version 4.0

<210> 1
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Event specific primer sequence designed for TC1507

<400> 1
gtagtactat agattatatt attcgtagag 30

<210> 2
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Event specific primer sequence designed for TC1507

<400> 2
gccatacaga actcaaaatc ttttccggag 30

<210> 3
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Event specific primer designed for TC1507.

<400> 3
tgtggtgttt gtggctctgt cctaa 25

<210> 4
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Event specific primer for TC1507.

<400> 4
agcacctttt cattctttca tatac 25

Copy provided by USPTO from the PACR Image Database on 07/20/2004

<210> 5
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Genomic DNA primer sequence

<400> 5
gacctcccca caggcatgat tgatc 25

<210> 6
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer in full length insert, 35S promoter to pat gene

<400> 6
tagaggacct aacagaactc gccgt 25

<210> 7
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer in full length insert, 35S promoter to pat gene

<400> 7
gagctggcaa ctcaaaatcc cttt 24

<210> 8
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer in full length insert, 35S promoter to pat gene

<400> 8
aaaatcttcg tcaacatggt ggagc 25

<210> 9
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer in full length insert, 35S promoter to pat gene

Copy provided by USPTO from the PACR Image Database on 07/26/2004

<400> 9
taatctcaac tggtctcctc tccgg 25

<210> 10
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA

<400> 10
cccctacccc accgacgttt at 22

<210> 11
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA

<400> 11
ttgattggca ggtccgtggg tc 22

<210> 12
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 12
cacaacggca cagaaacacg aa 22

<210> 13
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 13
gcgcacccac cggaacaaaa tg 22

<210> 14
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 14
tcctcgcatt aaatgctcct gc 22

Copy provided by USPTO from the PACR Image Database on 07/26/2004

<210> 15
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 15
cctggcacgc attgacgcat gt 22

<210> 16
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 16
aagccgccac cgtcttgagg acctt 25

<210> 17
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 17
gtcgaaccgt gcctccactt gctaa 25

<210> 18
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer - Zea mays genomic DNA.

<400> 18
agaaagggaa aatcagggcc aggac 25

<210> 19
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Cry1F sequence primer

<400> 19
ggctcggact cgacctttct at 22

<210> 20

Copy provided by USPTO from the PACR Image Database on 07/20/2004

<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Cry1F sequence primer

<400> 20
gcagttcttg aagaatgagt ga 22

<210> 21
<211> 2829
<212> DNA
<213> 5' flanking sequence of event TC1507

<400> 21
actagtttcc tagcccgcgt cgtgccccta ccccaccgac gtttatggaa ggtgccattc 60
cacggttctt cgtggccgcc cctaaggatg taaatggtcg gtaaaatccg gtaaatttcc 120
ggtaccgttt accagatttt tccagccgtt ttcggattta tcgggatata cagaaaacga 180
gacggaaacg gaataggttt tttttcgaaa acggtacggt aaacggtgag acaaacttac 240
cgtccgtttt cgtatttctc gggaaactct ggtatattcc cgtatttgtc ccgtattttc 300
ccgacccacg gacctgccaa tcaaccatca gccagtcagc ccatccccac agctatggcc 360
catggggcca tgttggccac atgcccacgc aacgcaaggc agtaaggctg gcagcctggc 420
acgcattgac gcatgtggac acacacagcc gccgcctgtt cgtgtttctg tgccgttgtg 480
cgagactgtg actgcgagtg gcggagtcgg cgaacggcga ggcgtctccg gagtctggac 540
tgcggctgtg gacagcgacg ctgtgacggc gactcggcga agccccaagc taccaagccc 600
ccaagtcccc atccatctct gcttctctgg tcatctcctt cccctggtcg atctgcaggc 660
gccagaccgg ccgaagcatc acgaaacgca ctaagacctc gaaggagtca aaccactcct 720
ccgaggcctc gggggctaca cccggcgggt gcgctcgcgc gcacccaccg gaacaaaatg 780
taaccgagaa aggtcggtcc ccttgcaaaa aaagtgcgac aaaagcctcc aagcgagtat 840
taacactcac tttgaggctc gggggctact gtcggggacc ataattaggg gtacccccaa 900
gactcctaat ctcagctggt aacccccatc agcacaaagc tgcaaaggcc tgatgggtgc 960
gattaagtca aggctcggtc cactcaaggg acacgatctc gcctcgcccg agcccagcct 1020
cgggcaaggg cggccgaccc cgaggattca cgtctcgccc gagggccccc tcaagcgacg 1080
ggcacacctt cggctcgccc gaggcccatt cttcgccgag aagcaacctt ggccagatcg 1140
ccacaccgac cgaccgtatc gcaggagcat ttaatgcgag gatcgcctga caccttatcc 1200
tgacgcgcgc tcttcagtcg acagagccga agtgaccgca atcacttcgc cgctccactg 1260
accgacctga caagaagaca gcgccgcctg cgtcgctccg actgctgtgc cactcgacag 1320
agtgaggctg acagcagcca agtccggcct cgggcgccat aggaagctcc gcctcgcccg 1380
accctagggc tcggactcgg cctcggctcc ggaagacgac gaactacgct tcgcccgacc 1440
ccagggcttg gactcagcct cggctccgga agacgacgaa ttccgcctcg cccgacccca 1500
gggctcggac tcggcctcgg ctccagaaga cgacgaactc cgcctcgccc gaccccaggg 1560
ctcggactca gcctcggctc cggaagacga cgaactccgc ctcgcccgac cccagggctc 1620
ggactcagcc tcggcctcag acgatggtct ccgcctcgcc cgacccgggg ctcggactcg 1680
acctttctat cggaccttgt cagatcctgt cttcgtccga ggaggctttg gcaatcctca 1740
ctatgtactc ggtcttaggg gagtggcctt tcaacaaact ggtacgaatc acacccgcac 1800
attcaggaac tccgggacca ttgactctct agatgagata ccacctcaag acaacagcgg 1860
cgcaccttgg aatgactact cccatgtgct gaatcatgtt acctttgtgc gctggccagg 1920
tgagatctca ggttccgact catggagagc accaatgttc tcttggacgc atcgtagcgc 1980
taccccaca aacaccattg atccagagag aatcactcat tcttcaagaa ctgcatatct 2040
tgccgagatc ctcatcccta aaggtacttg acaatagtat tattggagtc gatacacaac 2100
tcacaaaaaa tacaagaagt cgactaggtg gattggtccg agtgaagaga aaaaaaagcc 2160
atacagaact caaaatcttt tccggagata ttcattttcc tgaagaggcg gataagatat 2220
taggtggcag tttgatacca ccagaaagag aaaaaaaaga ttctaaggaa tcaaaaaaaa 2280
ggaaaaattg ggtttatgtt caacggaaaa aatttctcaa aagcaaggaa aagtattgtg 2340
gctatttatc tatccgtgca gctgatatgg ccgcggtttg tgatatcgtt aaccattaca 2400
ttgagacgtc tacagtgaac tttaggacag agccacaaac accacaagag tggattgatg 2460

Copy provided by USPTO from the PACR Image Database on 07/20/2004

atctagagag gttgcaagat agataccctt ggttggttgc tgaggttgag ggtgttgtgg 2520
ctggtattgc ttacgctggg ccctggaagg ctaggaaccc tcaacctcag caaccaacca 2580
atggtatcta tcttgcaacc tctctagatc atcaatccac tcttgtggtg tttgtggctc 2640
tgtcctaaag ttcactgtag acgtctcaat gtaatggtta acgatatcac aaaccgagag 2700
aagagggatc tcgaagcttc ggccggggcc catcgatatc cgcgggcatg cctgcagtgc 2760
agcgtgaccc ggtcgtgccc ctctctagag ataatgagca ttgcatgtct aagttataaa 2820
aaattacca 2829

<210> 22
<211> 2346
<212> DNA
<213> 3' flanking sequence of event TC1507

<400> 22
ctcactccgc ttgatcttgg caaagatatt tgacgcattt attagtatgt gttaattttc 60
atttgcagtg cagtattttc tattcgatct ttatgtaatt cgttacaatt aataaatatt 120
caaatcagat tattgactgt catttgtatc aaatcgtgtt taatggatat ttttattata 180
atattgatga tatctcaatc aaaacgtaga taataataat atttatttaa tatttttgcg 240
tcgcacagtg aaaatctata tgagattaca aaataccgac aacattattt aagaaacata 300
gacattaacc ctgagactgt tggacatcaa cgggtagatt ccttcatgca tagcacctca 360
ttcttgggga caaaagcacg gtttggccgt tccattgctg cacgaacgag ctttgctata 420
tcctcgggtt ggatcatctc atcaggtcca atcaaatttg tccaagaact catgttagtc 480
gcaacgaaac cggggcatat gtcgggtatc tcgagctcgc gaaagcttgg ctgcaggtcg 540
acggatcctt caacaaaagg gtacctgtac ccgaaaccga cacaggtggg taggtagaga 600
atacctaggg gcgcgagaca actctctcta aggaactcgg caaaatagcc ccgtaacttc 660
gggagaaggg gtgccccccg ctaacaataa acgaatacgg tttatgtatg gattccggta 720
aaataccggt actcgatttc ataagagtcg aataggaagt taagatgagg gtggtatcat 780
cataaaaatg gagtagtatc ctaaattata ctaatccacg tatgatatgt atgcctttcc 840
ttatcaaccg gaagtagtgc aaaaaaaatt ctatactgca ctgctctctt tttactgaga 900
aatgcaaaaa aataaaagtg aagtaagggt gccccataga tatttgatct tgcctcctgt 960
ccccccccc ctttttttcat caaaaatttc catgaaaaaa gaaaagatga atttgtccat 1020
tcattgaacc ctagttcggg actgacgggg ctcgaacccg cagcttccgc ctgttcctag 1080
ccttccaggg cccagcgtaa gcaataccag ccacagcacc ctcaacctca gcaaccaacc 1140
aagggtatct atcttgcaac ctctctagat catcaatcca ctcttgtggt gtttgtggct 1200
ctgtcctaaa gttcactgta gacgtctcaa tgtaatggtt aacgatatca caaaccgcgg 1260
aacacaagaa cgaaagcacc ttttcattct ttcatatact aggggttttt acttggaaaa 1320
gacaatgttc catactaaag gatagctgca gaagccgcca ccgtcttgag gaccttccgg 1380
ggagccagac cggtcgaacc gtgcctccac ttgctaagga gaaagggaaa atcagggcca 1440
ggacatacga aggaggagcc agaacgaaga tatcctaaga tacttactcg ctccgggcca 1500
tgatcaatca tgcctgtggg gaggtctctc gcacctcgat ccatgaaggt accaccgagg 1560
tctgccccgc cgccggcttc ggtaccgtcc tcgccttggg cgcccgaggc acccggggga 1620
tggactgccc aggcgcagcc acgacgaccc aaggatcacc ctcctgcgca gtcggcacga 1680
gcaatagttc tcggggaaca ggcagcttgg cctgactccc cggggtcacc tcaactacct 1740
cggccgaggg gtcaagtacc ccctcagtcc gcccccgctc ttcggaccgg gaccccgacg 1800
tcccggcccc ggataccgac ggcaccagcc cgctcggggg ctggcttgac gacccctggc 1860
ccagcctcag atctgggctg aggccgaggc aggcggccat gtcgtcgtct tcatcatcgt 1920
cttcatcatc gtcgtcgtca tcaggcgtct ccggcgacgg ctcccttggg agcccctccc 1980
tctcctgccg acgacgaagc ctttccaagg catcccgagc ccacgtccgc tcgtgggccc 2040
gagccttctt tgcgtccttc ttctccttcc tcttctccgc ggtgaccctc cgcgcagctc 2100
ggtccaccgc atcctccggg actggtggca gggaaggctt gtgatgccct acctcctgga 2160
gacagacgaa aagtctcagc tatgagaacc gagggcaatc tgacgcaaga aggaagaagg 2220
agcggatact caccagagac acgcacccgc gatcgggacg cattaagggc tgggaaaaag 2280
tgccggcctc taatttcgct accgtgccgt ccacccacct gtggaggtca tcgatgggaa 2340
ggggaa 2346

<210> 23
<211> 20
<212> DNA

Copy provided by USPTO from the PACR Image Database on 07/20/2004

<213> Artificial Sequence

<220>
<223> Event specific primer sequence designed for TC1507

<400> 23
cttcaaacaa gtgtgacaaa 20

<210> 24
<211> 11361
<212> DNA
<213> Artificial Sequence

<220>
<223> The sequence represents the transgenic insert in
maize line TC1507 as well as the sequence flanking
the insertion sites.

<400> 24
actagtttcc tagcccgcgt cgtgccccta ccccaccgac gtttatggaa ggtgccattc 60
cacggttctt cgtggccgcc cctaaggatg taaatggtcg gtaaaatccg gtaaatttcc 120
ggtaccgttt accagatttt tccagccgtt ttcggattta tcgggatata cagaaaacga 180
gacggaaacg gaataggttt tttttcgaaa acggtacggt aaacggtgag acaaacttac 240
cgtccgtttt cgtatttctc gggaaactct ggtatattcc cgtatttgtc ccgtattttc 300
ccgacccacg gacctgccaa tcaaccatca gccagtcagc ccatccccac agctatggcc 360
catggggcca tgttggccac atgcccacgc aacgcaaggc agtaaggctg gcagcctggc 420
acgcattgac gcatgtggac acacacagcc gccgcctgtt cgtgtttctg tgccgttgtg 480
cgagactgtg actgcgagtg gcggagtcgg cgaacggcga ggcgtctccg gagtctggac 540
tgcggctgtg gacagcgacg ctgtgacggc gactcggcga agccccaagc taccaagccc 600
ccaagtcccc atccatctct gcttctctgg tcatctcctt cccctggtcg atctgcaggc 660
gccagaccgg ccgaagcatc acgaaacgca ctaagacctc gaaggagtca aaccactcct 720
ccgaggcctc gggggctaca cccggcgggt gcgctcgcgc gcacccaccg gaacaaaatg 780
taaccgagaa aggtcggtcc ccttgcaaaa aaagtgcgac aaaagcctcc aagcgagtat 840
taacactcac tttgaggctc gggggctact gtcggggacc ataattaggg gtacccccaa 900
gactcctaat ctcagctggt aacccccatc agcacaaagc tgcaaaggcc tgatgggtgc 960
gattaagtca aggctcggtc cactcaaggg acacgatctc gcctcgcccg agcccagcct 1020
cgggcaaggg cggccgaccc cgaggattca cgtctcgccc gagggccccc tcaagcgacg 1080
ggcacacctt cggctcgccc gaggcccatt cttcgccgag aagcaacctt ggccagatcg 1140
ccacaccgac cgaccgtatc gcaggagcat ttaatgcgag gatcgcctga caccttatcc 1200
tgacgcgcgc tcttcagtcg acagagccga agtgaccgca atcacttcgc cgctccactg 1260
accgacctga caagaagaca gcgccgcctg cgtcgctccg actgctgtgc cactcgacag 1320
agtgaggctg acagcagcca agtccggcct cgggcgccat aggaagctcc gcctcgcccg 1380
accctagggc tcggactcgg cctcggctcc ggaagacgac gaactacgct tcgcccgacc 1440
ccagggcttg gactcagcct cggctccgga agacgacgaa ttccgcctcg cccgacccca 1500
gggctcggac tcggcctcgg ctccagaaga cgacgaactc cgcctcgccc gaccccaggg 1560
ctcggactca gcctcggctc cggaagacga cgaactccgc ctcgcccgac cccagggctc 1620
ggactcagcc tcggcctcag acgatggtct ccgcctcgcc cgacccgggg ctcggactcg 1680
acctttctat cggaccttgt cagatcctgt cttcgtccga ggaggctttg gcaatcctca 1740
ctatgtactc ggtcttaggg gagtggcctt tcaacaaact ggtacgaatc acacccgcac 1800
attcaggaac tccgggacca ttgactctct agatgagata ccacctcaag acaacagcgg 1860
cgcaccttgg aatgactact cccatgtgct gaatcatgtt acctttgtgc gctggccagg 1920
tgagatctca ggttccgact catggagagc accaatgttc tcttggacgc atcgtagcgc 1980
taccccaca aacaccattg atccagagag aatcactcat tcttcaagaa ctgcatatct 2040
tgccgagatc ctcatcccta aaggtacttg acaatagtat tattggagtc gatacacaac 2100
tcacaaaaaa tacaagaagt cgactaggtg gattggtccg agtgaagaga aaaaaaagcc 2160
atacagaact caaaatcttt tccggagata ttcattttcc tgaagaggcg gataagatat 2220
taggtggcag tttgatacca ccagaaagag aaaaaaaaga ttctaaggaa tcaaaaaaaa 2280
ggaaaaattg ggtttatgtt caacggaaaa aatttctcaa aagcaaggaa aagtattgtg 2340

Copy provided by USPTO from the PACR Image Database on 07/26/2004

gctatttatc tatccgtgca gctgatatgg ccgcggtttg tgatatcgtt aaccattaca 2400
ttgagacgtc tacagtgaac tttaggacag agccacaaac accacaagag tggattgatg 2460
atctagagag gttgcaagat agataccctt ggttggttgc tgaggttgag ggtgttgtgg 2520
ctggtattgc ttacgctggg ccctggaagg ctaggaaccc tcaacctcag caaccaacca 2580
atggtatcta tcttgcaacc tctctagatc atcaatccac tcttgtggtg tttgtggctc 2640
tgtcctaaag ttcactgtag acgtctcaat gtaatggtta acgatatcac aaaccgagag 2700
aagagggatc tcgaagcttc ggccggggcc catcgatatc cgcgggcatg cctgcagtgc 2760
agcgtgaccc ggtcgtgccc ctctctagag ataatgagca ttgcatgtct aagttataaa 2820
aaattaccac aactggaaga gcggttaccc ggaccgaagc ttcggccggg gcccatcgat 2880
atccgcgggc atgcctgcag tgcagcgtga cccggtcgtg cccctctcta gagataatga 2940
gcattgcatg tctaagttat aaaaaattac cacatatttt ttttgtcaca cttgtttgaa 3000
gtgcagttta tctatcttta tacatatatt taaactttac tctacgaata atataatcta 3060
tagtactaca ataatatcag tgttttagag aatcatataa atgaacagtt agacatggtc 3120
taaaggacaa ttgagtattt tgacaacagg actctacagt tttatctttt tagtgtgcat 3180
gtgttctcct tttttttgc aaatagcttc acctatataa tacttcatcc attttattag 3240
tacatccatt tagggtttag ggttaatggt ttttatagac taatttttt agtacatcta 3300
ttttattcta ttttagcctc taaattaaga aaactaaaac tctattttag tttttttatt 3360
taataattta gatataaaat agaataaaat aaagtgacta aaaattaaac aaatacccct 3420
taagaaatta aaaaaactaa ggaaacattt ttcttgtttc gagtagataa tgccagcctg 3480
ttaaacgccg tcgacgagtc taacggacac caaccagcga accagcagcg tcgcgtcggg 3540
ccaagcgaag cagacggcac ggcatctctg tcgctgcctc tggacccctc tcgagagttc 3600
cgctccaccg ttggacttgc tccgctgtcg gcatccagaa attgcgtggc ggagcggcag 3660
acgtgagccg gcacggcagg cggcctcctc ctcctctcac ggcacggcag ctacgggggа 3720
ttcctttccc accgctcctt cgctttcctt tcctcgcccg ccgtaataaa tagacacccc 3780
ctccacaccc tctttcccca acctcgtgtt gttcggagcg cacacacaca caaccagatc 3840
tcccccaaat ccacccgtcg gcacctccgc ttcaaggtac gccgctcgtc ctcccccccc 3900
ccccctctct accttctcta gatcggcgtt ccggtccatg gttagggccc ggtagttcta 3960
cttctgttca tgtttgtgtt agatccgtgt ttgtgttaga tccgtgctgc tagcgttcgt 4020
acacggatgc gacctgtacg tcagacacgt tctgattgct aacttgccag tgtttctctt 4080
tggggaatcc tgggatggct ctagccgttc cgcagacggg atcgatttca tgatttttt 4140
tgtttcgttg catagggttt ggtttgccct tttcctttat ttcaatatat gccgtgcact 4200
tgtttgtcgg gtcatctttt catgcttttt tttgtcttgg ttgtgatgat gtggtctggt 4260
tgggcggtcg ttctagatcg gagtagaatt ctgtttcaaa ctacctggtg gatttattaa 4320
ttttggatct gtatgtgtgt gccatacata ttcatagtta cgaattgaag atgatggatg 4380
gaaatatcga tctaggatag gtatacatgt tgatgcgggt tttactgatg catatacaga 4440
gatgcttttt gttcgcttgg ttgtgatgat gtggtgtggt tgggcggtcg ttcattcgtt 4500
ctagatcgga gtagaatact gtttcaaact acctggtgta tttattaatt ttggaactgt 4560
atgtgtgtgt catacatctt catagttacg agtttaagat ggatggaaat atcgatctag 4620
gataggtata catgttgatg tgggttttac tgatgcatat acatgatggc atatgcagca 4680
tctattcata tgctctaacc ttgagtacct atctattata ataaacaagt atgttttata 4740
attattttga tcttgatata cttggatgat ggcatatgca gcagctatat gtggattttt 4800
ttagccctgc cttcatacgc tatttatttg cttggtactg tttcttttgt cgatgctcac 4860
cctgttgttt ggtgttactt ctgcaggtcg actctagagg atccaacaat ggagaacaac 4920
atacagaatc agtgcgtccc ctacaactgc ctcaacaatc ctgaagtaga gattctcaac 4980
gaagagaggt cgactggcag attgccgtta gacatctccc tgtcccttac acgtttcctg 5040
ttgtctgagt ttgttccagg tgtgggagtt gcgtttggcc tcttcgacct catctggggc 5100
ttcatcactc catctgattg gagcctcttt cttctccaga ttgaacagtt gattgaacaa 5160
aggattgaga ccttggaaag gaatcgggcc atcactaccc ttcgtggctt agcagacagc 5220
tatgagatct acattgaagc actaagagag tgggaagcca atcctaacaa tgcccaactg 5280
agagaagatg tgcgtatacg ctttgctaac acagatgatg ctttgatcac agccatcaac 5340
aacttcaccc ttaccagctt cgagatccct cttctctcgg tctatgttca agctgctaac 5400
ctgcacttgt cactactgcg cgacgctgtg tcgtttgggc aaggttgggg actggacata 5460
gctactgtca acaatcacta caacagactc atcaatctga ttcatcgata cacgaaacat 5520
tgtttggata cctacaatca gggattggag aacctgagag gtactaacac tcgccaatgg 5580
gccaggttca atcagttcag gagagacctt acacttactg tgttagacat agttgctctc 5640
tttccgaact acgatgttcg tacctatccg attcaaacgt catcccaact tacaagggag 5700
atctacacca gttcagtcat tgaagactct ccagtttctg cgaacatacc caatggtttc 5760
aacagggctg agtttggagt cagaccaccc catctcatgg acttcatgaa ctctttgttt 5820

Copy provided by USPTO from the PACR Image Database on 07/26/2004

gtgactgcag agactgttag atcccaaact gtgtggggag gacacttagt tagctcacgc 5880
aacacggctg gcaatcgtat caactttcct agttacgggg tcttcaatcc cgggggcgcc 5940
atctggattg cagatgaaga tccacgtcct ttctatcgga ccttgtcaga tcctgtcttc 6000
gtccgaggag gctttggcaa tcctcactat gtactcggtc ttaggggagt ggcctttcaa 6060
caaactggta cgaatcacac ccgcacattc aggaactccg ggaccattga ctctctagat 6120
gagataccac ctcaagacaa cagcggcgca ccttggaatg actactccca tgtgctgaat 6180
catgttacct ttgtgcgctg gccaggtgag atctcaggtt ccgactcatg gagagcacca 6240
atgttctctt ggacgcatcg tagcgctacc cccacaaaca ccattgatcc agagagaatc 6300
actcagattc ccttggtgaa ggcacacaca cttcagtcag gaactacagt tgtaagaggg 6360
ccggggttca cgggaggaga cattcttcga cgcactagtg gaggaccatt cgcgtacacc 6420
attgtcaaca tcaatgggca acttccccaa aggtatcgtg ccaggatacg ctatgcctct 6480
actaccaatc taagaatcta cgttacggtt gcaggtgaac ggatctttgc tggtcagttc 6540
aacaagacaa tggataccgg tgatccactt acattccaat ctttctccta cgccactatc 6600
aacaccgcgt tcacctttcc aatgagccag agcagtttca cagtaggtgc tgataccttc 6660
agttcaggca acgaagtgta cattgacagg tttgagttga ttccagttac tgccacactc 6720
gagtaaggat ccgtcgacct gcagccaagc tttcgcgagc tcgagatccc cgacatatgc 6780
cccggtttcg ttgcgactaa catgagttct tggacaaatt tgattggacc tgatgagatg 6840
atccaacccg aggatatagc aaagctcgtt cgtgcagcaa tggaacggcc aaaccgtgct 6900
tttgtcccca agaatgaggt gctatgcatg aaggaatcta cccgttgatg tccaacagtc 6960
tcagggttaa tgtctatgta tcttaaataa tgttgtcggt attttgtaat ctcatataga 7020
ttttcactgt gcgacgcaaa aatattaaat aaatattatt attatctacg ttttgattga 7080
gatatcatca atattataat aaaaatatcc attaaacacg atttgataca aatgacagtc 7140
aataatctga tttgaatatt tattaattgt aacgaattac ataaagatcg aatagaaaat 7200
actgcactgc aaatgaaaat taacacatac taataaatgc gtcaaatatc tttgccaaga 7260
tcaagcggag tgagggcctc atatccggtc tcagttacaa gcacggtatc cccgaagcgc 7320
gctccaccaa tgccctcgac atagatgccg ggctcgacgc tgaggacatt gcctaccttg 7380
agcatggtct cagcgccggc tttaagctca atcccatccc aatctgaata tcctatcccg 7440
cgcccagtcc ggtgtaagaa cgggtctgtc catccacctc tgttgggaat tccggtccgg 7500
gtcacctttg tccaccaaga tggaactgcg gccgcggacc gaattcccat ggagtcaaag 7560
attcaaatag aggacctaac agaactcgcc gtaaagactg gcgaacagtt catacagagt 7620
ctcttacgac tcaatgacaa gaagaaaatc ttcgtcaaca tggtggagca cgacacgctt 7680
gtctactcca aaaatatcaa agatacagtc tcagaagacc aaagggcaat tgagactttt 7740
caacaaaggg taatatccgg aaacctcctc ggattccatt gcccagctat ctgtcacttt 7800
attgtgaaga tagtggaaaa ggaaggtggc tcctacaaat gccatcattg cgataaagga 7860
aaggccatcg ttgaagatgc ctctgccgac agtggtccca aagatggacc cccacccacg 7920
aggagcatcg tggaaaaaga agacgttcca accacgtctt caaagcaagt ggattgatgt 7980
gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 8040
tctatataag gaagttcatt tcatttggag aggacagggt acccggggat ccaccatgtc 8100
tccggagagg agaccagttg agattaggcc agctacagca gctgatatgg ccgcggtttg 8160
tgatatcgtt aaccattaca ttgagacgtc tacagtgaac tttaggacag agccacaaac 8220
accacaagag tggattgatg atctagagag gttgcaagat agataccctt ggttggttgc 8280
tgaggttgag ggtgttgtgg ctggtattgc ttacgctggg ccctggaagg ctaggaacgc 8340
ttacgattgg acagttgaga gtactgttta cgtgtcacat aggcatcaaa ggttgggcct 8400
aggatccaca ttgtacacac atttgcttaa gtctatggag gcgcaaggtt ttaagtctgt 8460
ggttgctgtt ataggccttc caaacgatcc atctgttagg ttgcatgagg ctttgggata 8520
cacagcccgg ggtacattgc gcgcagctgg atacaagcat ggtggatggc atgatgttgg 8580
tttttggcaa agggattttg agttgccagc tcctccaagg ccagttaggc cagttaccca 8640
gatctgagtc gacctgcagg catgcccgct gaaatcacca gtctctctct acaaatctat 8700
ctctctctat aataatgtgt gagtagttcc cagataaggg aattagggtt cttatagggt 8760
ttcgctcatg tgttgagcat ataagaaacc cttagtatgt atttgtattt gtaaaatact 8820
tctatcaata aaatttctaa ttcctaaaac caaaatccag tggcgagctc gaattcgagc 8880
tcgagcccgg gtggatcctc tagagtcgac ctgcagaagc ttcggtccgg cgcgcctcta 8940
gttgaagaca cgttcatgtc ttcatcgtaa gaagacactc agtagtcttc ggccagaatg 9000
gcctaactca aggccctcac tccgcttgat cttggcaaag atatttgacg catttattag 9060
tatgtgttaa ttttcatttg cagtgcagta ttttctattc gatctttatg taattcgtta 9120
caattaataa atattcaaat cagattattg actgtcattt gtatcaaatc gtgtttaatg 9180
gatatttta ttataatatt gatgatatct caatcaaaac gtagataata ataatattta 9240
tttaatattt ttgcgtcgca cagtgaaaat ctatatgaga ttacaaaata ccgacaacat 9300

Copy provided by USPTO from the PACR Image Database on 07/26/2004

tatttaagaa acatagacat taaccctgag actgttggac atcaacgggt agattccttc 9360
atgcatagca cctcattctt ggggacaaaa gcacggtttg gccgttccat tgctgcacga 9420
acgagctttg ctatatcctc gggttggatc atctcatcag gtccaatcaa atttgtccaa 9480
gaactcatgt tagtcgcaac gaaaccgggg catatgtcgg gtatctcgag ctcgcgaaag 9540
cttggctgca ggtcgacgga tccttcaaca aaagggtacc tgtacccgaa accgacacag 9600
gtgggtaggt agagaatacc taggggcgcg agacaactct ctctaaggaa ctcggcaaaa 9660
tagccccgta acttcgggag aaggggtgcc ccccgctaac aataaacgaa tacggtttat 9720
gtatggattc cggtaaaata ccggtactcg atttcataag agtcgaatag gaagttaaga 9780
tgagggtggt atcatcataa aaatggagta gtatcctaaa ttatactaat ccacgtatga 9840
tatgtatgcc tttccttatc aaccggaagt agtgcaaaaa aaattctata ctgcactgct 9900
ctctttttac tgagaaatgc aaaaaaataa aagtgaagta agggtgcccc atagatattt 9960
gatcttgcct cctgtccccc ccccccTTTT ttcatcaaaa atttccatga aaaaagaaaa 10020
gatgaatttg tccattcatt gaaccctagt tcgggactga cggggctcga acccgcagct 10080
tccgcctgtt cctagccttc cagggcccag cgtaagcaat accagccaca gcaccctcaa 10140
cctcagcaac caaccaaggg tatctatctt gcaacctctc tagatcatca atccactctt 10200
gtggtgtttg tggctctgtc ctaaagttca ctgtagacgt ctcaatgtaa tggttaacga 10260
tatcacaaac cgcggaacac aagaacgaaa gcacctTTTc attctttcat atactagggg 10320
tttttacttg gaaaagacaa tgttccatac taaaggatag ctgcagaagc cgccaccgtc 10380
ttgaggacct tccggggagc cagaccggtc gaaccgtgcc tccacttgct aaggagaaag 10440
ggaaaatcag ggccaggaca tacgaaggag gagccagaac gaagatatcc taagatactt 10500
actcgctccg ggccatgatc aatcatgcct gtggggaggt ctctcgcacc tcgatccatg 10560
aaggtaccac cgaggtctgc cccgccgccg gcttcggtac cgtcctcgcc ttgggcgccc 10620
gaggcacccg ggggatggac tgcccaggcg cagccacgac gacccaagga tcaccctcct 10680
gcgcagtcgg cacgagcaat agttctcggg gaacaggcag cttggcctga ctccccgggg 10740
tcacctcaac tacctcggcc gaggggtcaa gtacccccTc agtccgcccc cgctcttcgg 10800
accgggaccc cgacgtcccg gccccggata ccgacggcac cagcccgctc gggggctggc 10860
ttgacgaccc ctggcccagc ctcagatctg ggctgaggcc gaggcaggcg gccatgtcgt 10920
cgtcttcatc atcgtcttca tcatcgtcgt cgtcatcagg cgtctccggc gacggctccc 10980
ttgggagccc ctccctctcc tgccgacgac gaagcctttc caaggcatcc cgagcccacg 11040
tccgctcgtg ggcccgagcc ttctttgcgt ccttcttctc cttcctcttc tccgcggtga 11100
ccctccgcgc agctcggtcc accgcatcct ccgggactgg tggcagggaa ggcttgtgat 11160
gccctacctc ctggagacag acgaaaagtc tcagctatga gaaccgaggg caatctgacg 11220
caagaaggaa gaaggagcgg atactcacca gagacacgca cccgcgatcg ggacgcatta 11280
agggctggga aaaagtgccg gcctctaatt tcgctaccgt gccgtccacc cacctgtgga 11340
ggtcatcgat gggaagggga a 11361

<210> 25
<211> 6235
<212> DNA
<213> Artificial Sequence

<220>
<223> The sequence represents the DNA molecule used to
transform maize line TC1507. This sequence
represents insert PHI8999A.

<400> 25
aaacgctctt caactggaag agcggttacc cggaccgaag cttcggccgg ggcccatcga 60
tatccgcggg catgcctgca gtgcagcgtg acccggtcgt gcccctctct agagataatg 120
agcattgcat gtctaagtta taaaaaatta ccacatattt tttttgtcac acttgtttga 180
agtgcagttt atctatcttt atacatatat ttaaacttta ctctacgaat aatataatct 240
atagtactac aataatatca gtgttttaga gaatcatata aatgaacagt tagacatggt 300
ctaaaggaca attgagtatt ttgacaacag gactctacag ttttatcttt ttagtgtgca 360
tgtgttctcc tttttttttg caaatagctt cacctatata atacttcatc cattttatta 420
gtacatccat ttagggttta gggttaatgg tttttataga ctaatttttt tagtacatct 480
attttattct attttagcct ctaaattaag aaaactaaaa ctctatttta gtttttttat 540

Copy provided by USPTO from the PACR Image Database on 07/26/2004

ttaataattt agatataaaa tagaataaaa taaagtgact aaaaattaaa caaataccct 600
ttaagaaatt aaaaaaacta aggaaacatt tttcttgttt cgagtagata atgccagcct 660
gttaaacgcc gtcgacgagt ctaacggaca ccaaccagcg aaccagcagc gtcgcgtcgg 720
gccaagcgaa gcagacggca cggcatctct gtcgctgcct ctggacccct ctcgagagtt 780
ccgctccacc gttggacttg ctccgctgtc ggcatccaga aattgcgtgg cggagcggca 840
gacgtgagcc ggcacggcag gcggcctcct cctcctctca cggcacggca gctacggggg 900
attcctttcc caccgctcct tcgctttccc ttcctcgccc gccgtaataa atagacaccc 960
cctccacacc ctctttcccc aacctcgtgt tgttcggagc gcacacacac acaaccagat 1020
ctcccccaaa tccacccgtc ggcacctccg cttcaaggta cgccgctcgt cctccccccc 1080
ccccctctc taccttctct agatcggcgt tccggtccat ggttagggcc cggtagttct 1140
acttctgttc atgtttgtgt tagatccgtg tttgtgttag atccgtgctg ctagcgttcg 1200
tacacggatg cgacctgtac gtcagacacg ttctgattgc taacttgcca gtgtttctct 1260
ttggggaatc ctgggatggc tctagccgtt ccgcagacgg gatcgatttc atgatttttt 1320
ttgtttcgtt gcatagggtt tggtttgccc ttttccttta tttcaatata tgccgtgcac 1380
ttgtttgtcg ggtcatcttt tcatgctttt ttttgtcttg gttgtgatga tgtggtctgg 1440
ttgggcggtc gttctagatc ggagtagaat tctgtttcaa actacctggt ggatttatta 1500
attttggatc tgtatgtgtg tgccatacat attcatagtt acgaattgaa gatgatggat 1560
ggaaatatcg atctaggata ggtatacatg ttgatgcggg ttttactgat gcatatacag 1620
agatgctttt tgttcgcttg gttgtgatga tgtggtgtgg ttgggcggtc gttcattcgt 1680
tctagatcgg agtagaatac tgtttcaaac tacctggtgt atttattaat tttggaactg 1740
tatgtgtgtg tcatacatct tcatagttac gagtttaaga tggatggaaa tatcgatcta 1800
ggataggtat acatgttgat gtgggtttta ctgatgcata tacatgatgg catatgcagc 1860
atctattcat atgctctaac cttgagtacc tatctattat aataaacaag tatgttttat 1920
aattattttg atcttgatat acttggatga tggcatatgc agcagctata tgtggatttt 1980
tttagccctg ccttcatacg ctatttattt gcttggtact gtttcttttg tcgatgctca 2040
ccctgttgtt tggtgttact tctgcaggtc gactctagag gatccaacaa tggagaacaa 2100
catacagaat cagtgcgtcc cctacaactg cctcaacaat cctgaagtag agattctcaa 2160
cgaagagagg tcgactggca gattgccgtt agacatctcc ctgtccctta cacgtttcct 2220
gttgtctgag tttgttccag gtgtgggagt tgcgtttggc ctcttcgacc tcatctgggg 2280
cttcatcact ccatctgatt ggagcctctt tcttctccag attgaacagt tgattgaaca 2340
aaggattgag accttggaaa ggaatcgggc catcactacc cttcgtggct tagcagacag 2400
ctatgagatc tacattgaag cactaagaga gtgggaagcc aatcctaaca atgcccaact 2460
gagagaagat gtgcgtatac gctttgctaa cacagatgat gctttgatca cagccatcaa 2520
caacttcacc cttaccagct tcgagatccc tcttctctcg gtctatgttc aagctgctaa 2580
cctgcacttg tcactactgc gcgacgctgt gtcgtttggg caaggttggg gactggacat 2640
agctactgtc aacaatcact acaacagact catcaatctg attcatcgat acacgaaaca 2700
ttgtttggat acctacaatc agggattgga gaacctgaga ggtactaaca ctcgccaatg 2760
ggccaggttc aatcagttca ggagagacct tacacttact gtgttagaca tagttgctct 2820
ctttccgaac tacgatgttc gtacctatcc gattcaaacg tcatcccaac ttacaaggga 2880
gatctacacc agttcagtca ttgaagactc tccagtttct gcgaacatac ccaatggttt 2940
caacagggct gagtttggag tcagaccacc ccatctcatg gacttcatga actctttgtt 3000
tgtgactgca gagactgtta gatcccaaac tgtgtgggga ggacacttag ttagctcacg 3060
caacacggct ggcaatcgta tcaactttcc tagttacggg gtcttcaatc ccgggggcgc 3120
catctggatt gcagatgaag atccacgtcc tttctatcgg accttgtcag atcctgtctt 3180
cgtccgagga ggctttggca atcctcacta tgtactcggt cttaggggag tggcctttca 3240
acaaactggt acgaatcaca cccgcacatt caggaactcc gggaccattg actctctaga 3300
tgagatacca cctcaagaca acagcggcgc accttggaat gactactccc atgtgctgaa 3360
tcatgttacc tttgtgcgct ggccaggtga gatctcaggt tccgactcat ggagagcacc 3420
aatgttctct tggacgcatc gtagcgctac ccccacaaac accattgatc cagagagaat 3480
cactcagatt cccttggtga aggcacacac acttcagtca ggaactacag ttgtaagagg 3540
gccggggttc acgggaggag acattcttcg acgcactagt ggaggaccat tcgcgtacac 3600
cattgtcaac atcaatgggc aacttcccca aaggtatcgt gccaggatac gctatgcctc 3660
tactaccaat ctaagaatct acgttacggt tgcaggtgaa cggatctttg ctggtcagtt 3720
caacaagaca atggataccg gtgatccact tacattccaa tctttctcct acgccactat 3780
caacaccgcg ttcacctttc caatgagcca gagcagtttc acagtaggtg ctgatacctt 3840
cagttcaggc aacgaagtgt acattgacag gtttgagttg attccagtta ctgccacact 3900
cgagtaagga tccgtcgacc tgcagccaag ctttcgcga gctcgagatc cccgacatat 3960
gccccggttt cgttgcgact aacatgagtt cttggacaaa tttgattgga cctgatgaga 4020

Copy provided by USPTO from the PACR Image Database on 07/20/2004

tgatccaacc cgaggatata gcaaagctcg ttcgtgcagc aatggaacgg ccaaaccgtg 4080
cttttgtccc caagaatgag gtgctatgca tgaaggaatc tacccgttga tgtccaacag 4140
tctcagggtt aatgtctatg tatcttaaat aatgttgtcg gtattttgta atctcatata 4200
gattttcact gtgcgacgca aaaatattaa ataaatatta ttattatcta cgttttgatt 4260
gagatatcat caatattata ataaaaatat ccattaaaca cgatttgata caaatgacag 4320
tcaataatct gatttgaata tttattaatt gtaacgaatt acataaagat cgaatagaaa 4380
atactgcact gcaaatgaaa attaacacat actaataaat gcgtcaaata tctttgccaa 4440
gatcaagcgg agtgagggcc tcatatccgg tctcagttac aagcacggta tccccgaagc 4500
gcgctccacc aatgccctcg acatagatgc cgggctcgac gctgaggaca ttgcctacct 4560
tgagcatggt ctcagcgccg gctttaagct caatcccatc ccaatctgaa tatcctatcc 4620
cgcgcccagt ccggtgtaag aacgggtctg tccatccacc tctgttggga attccggtcc 4680
gggtcacctt tgtccaccaa gatggaactg cggccgcgga ccgaattccc atggagtcaa 4740
agattcaaat agaggaccta acagaactcg ccgtaaagac tggcgaacag ttcatacaga 4800
gtctcttacg actcaatgac aagaagaaaa tcttcgtcaa catggtggag cacgacacgc 4860
ttgtctactc caaaaatatc aaagatacag tctcagaaga ccaaagggca attgagactt 4920
ttcaacaaag ggtaatatcc ggaaacctcc tcggattcca ttgcccagct atctgtcact 4980
ttattgtgaa gatagtggaa aaggaaggtg gctcctacaa atgccatcat tgcgataaag 5040
gaaaggccat cgttgaagat gcctctgccg acagtggtcc caaagatgga cccccaccca 5100
cgaggagcat cgtggaaaaa gaagacgttc caaccacgtc ttcaaagcaa gtggattgat 5160
gtgatatctc cactgacgta agggatgacg cacaatccca ctatccttcg caagacccct 5220
cctctatata aggaagttca tttcatttgg agaggacagg gtacccgggg atccaccatg 5280
tctccggaga ggagaccagt tgagattagg ccagctacag cagctgatat ggccgcggtt 5340
tgtgatatcg ttaaccatta cattgagacg tctacagtga actttaggac agagccacaa 5400
acaccacaag agtggattga tgatctagag aggttgcaag atagataccc ttggttggtt 5460
gctgaggttg agggtgttgt ggctggtatt gcttacgctg ggccctggaa ggctaggaac 5520
gcttacgatt ggacagttga gagtactgtt tacgtgtcac ataggcatca aaggttgggc 5580
ctaggatcca cattgtacac acatttgctt aagtctatgg aggcgcaagg ttttaagtct 5640
gtggttgctg ttataggcct tccaaacgat ccatctgtta ggttgcatga ggctttggga 5700
tacacagccc ggggtacatt gcgcgcagct ggatacaagc atggtggatg gcatgatgtt 5760
ggtttttggc aaagggattt tgagttgcca gctcctccaa ggccagttag gccagttacc 5820
cagatctgag tcgacctgca ggcatgccgc tgaaatcacc agtctctctc tacaaatcta 5880
tctctctcta taataatgtg tgagtagttc ccagataagg gaattagggt tcttataggg 5940
tttcgctcat gtgttgagca tataagaaac ccttagtatg tatttgtatt tgtaaaatac 6000
ttctatcaat aaaatttcta attcctaaaa ccaaaatcca gtggcgagct cgaattcgag 6060
ctcgagcccg ggtggatcct ctagagtcga cctgcagaag cttcggtccg gcgcgcctct 6120
agttgaagac acgttcatgt cttcatcgta agaagacact cagtagtctt cggccagaat 6180
ggcctaactc aaggccatcg tggcctcttg ctcttcagga tgaagagcta tgttt 6235

<210> 26
<211> 3830
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence that represents part of the PHI8999A
insert as well as flanking sequence 5' to the
insert.

<400> 26
actagtttcc tagcccgcgt cgtgccccta ccccaccgac gtttatggaa ggtgccattc 60
cacggttctt cgtggccgcc cctaaggatg taaatggtcg gtaaaatccg gtaaatttcc 120
ggtaccgttt accagatttt tccagccgtt ttcggattta tcgggatata cagaaaacga 180
gacggaaacg gaataggttt tttttcgaaa acggtacggt aaacggtgag acaaacttac 240
cgtccgtttt cgtatttctc gggaaactct ggtatattcc cgtatttgtc ccgtattttc 300
ccgacccacg gacctgccaa tcaaccatca gccagtcagc ccatccccac agctatggcc 360
catggggcca tgttggccac atgcccacgc aacgcaaggc agtaaggctg gcagcctggc 420

Copy provided by USPTO from the PACR Image Database on 07/26/2004

```
acgcattgac gcatgtggac acacacagcc gccgcctgtt cgtgtttctg tgccgttgtg 480
cgagactgtg actgcgagtg gcggagtcgg cgaacggcga ggcgtctccg gagtctggac 540
tgcggctgtg gacagcgacg ctgtgacggc gactcggcga agccccaagc taccaagccc 600
ccaagtcccc atccatctct gcttctctgg tcatctcctt cccctggtcg atctgcaggc 660
gccagaccgg ccgaagcatc acgaaacgca ctaagacctc gaaggagtca aaccactcct 720
ccgaggcctc gggggctaca cccggcgggt gcgctcgcgc gcacccaccg gaacaaaatg 780
taaccgagaa aggtcggtcc ccttgcaaaa aaagtgcgac aaaagcctcc aagcgagtat 840
taacactcac tttgaggctc gggggctact gtcggggacc ataattaggg gtacccccaa 900
gactcctaat ctcagctggt aacccccatc agcacaaagc tgcaaaggcc tgatgggtgc 960
gattaagtca aggctcggtc cactcaaggg acacgatctc gcctcgcccg agcccagcct 1020
cgggcaaggg cggccgaccc cgaggattca cgtctcgccc gagggccccc tcaagcgacg 1080
ggcacacctt cggctcgccc gaggcccatt cttcgccgag aagcaacctt ggccagatcg 1140
ccacaccgac cgaccgtatc gcaggagcat ttaatgcgag gatcgcctga caccttatcc 1200
tgacgcgcgc tcttcagtcg acagagccga agtgaccgca atcacttcgc cgctccactg 1260
accgacctga caagaagaca gcgccgcctg cgtcgctccg actgctgtgc cactcgacag 1320
agtgaggctg acagcagcca agtccggcct cgggcgccat aggaagctcc gcctcgcccg 1380
accctagggc tcggactcgg cctcggctcc ggaagacgac gaactacgct tcgcccgacc 1440
ccagggcttg gactcagcct cggctccgga agacgacgaa ttccgcctcg cccgacccca 1500
gggctcggac tcggcctcgg ctccagaaga cgacgaactc cgcctcgccc gaccccaggg 1560
ctcggactca gcctcggctc cggaagacga cgaactccgc ctcgcccgac cccagggctc 1620
ggactcagcc tcggcctcag acgatggtct ccgcctcgcc cgacccgggg ctcggactcg 1680
acctttctat cggaccttgt cagatcctgt cttcgtccga ggaggctttg gcaatcctca 1740
ctatgtactc ggtcttaggg gagtggcctt tcaacaaact ggtacgaatc acacccgcac 1800
attcaggaac tccgggacca ttgactctct agatgagata ccacctcaag acaacagcgg 1860
cgcaccttgg aatgactact cccatgtgct gaatcatgtt acctttgtgc gctggccagg 1920
tgagatctca ggttccgact catggagagc accaatgttc tcttggacgc atcgtagcgc 1980
taccccaca aacaccattg atccagagag aatcactcat tcttcaagaa ctgcatatct 2040
tgccgagatc ctcatcccta aaggtacttg acaatagtat tattggagtc gatacacaac 2100
tcacaaaaaa tacaagaagt cgactaggtg gattggtccg agtgaagaga aaaaaaagcc 2160
atacagaact caaaatcttt tccggagata ttcattttcc tgaagaggcg gataagatat 2220
taggtggcag tttgatacca ccagaaagag aaaaaaaaga ttctaaggaa tcaaaaaaaa 2280
ggaaaaattg ggtttatgtt caacggaaaa aatttctcaa aagcaaggaa aagtattgtg 2340
gctatttatc tatccgtgca gctgatatgg ccgcggtttg tgatatcgtt aaccattaca 2400
ttgagacgtc tacagtgaac tttaggacag agccacaaac accacaagag tggattgatg 2460
atctagagag gttgcaagat agataccctt ggttggttgc tgaggttgag ggtgttgtgg 2520
ctggtattgc ttacgctggg ccctggaagg ctaggaaccc tcaacctcag caaccaacca 2580
atggtatcta tcttgcaacc tctctagatc atcaatccac tcttgtggtg tttgtggctc 2640
tgtcctaaag ttcactgtag acgtctcaat gtaatggtta acgatatcac aaaccgagag 2700
aagagggatc tcgaagcttc ggccggggcc catcgatatc cgcgggcatg cctgcagtgc 2760
agcgtgaccc ggtcgtgccc ctctctagag ataatgagca ttgcatgtct aagttataaa 2820
aaattaccac aactggaaga gcggttaccc ggaccgaagc ttcggccggg gcccatcgat 2880
atccgcgggc atgcctgcag tgcagcgtga cccggtcgtg cccctctcta gagataatga 2940
gcattgcatg tctaagttat aaaaaattac cacatatttt ttttgtcaca cttgtttgaa 3000
gtgcagttta tctatcttta tacatatatt taaactttac tctacgaata atataatcta 3060
tagtactaca ataatatcag tgttttagag aatcatataa atgaacagtt agacatggtc 3120
taaaggacaa ttgagtattt tgacaacagg actctacagt tttatctttt tagtgtgcat 3180
gtgttctcct tttttttgc aaatagcttc acctatataa tacttcatcc attttattag 3240
tacatccatt tagggtttag ggttaatggt ttttatagac taatttttt agtacatcta 3300
ttttattcta ttttagcctc taaattaaga aaactaaaac tctattttag tttttttatt 3360
taataattta gatataaaat agaataaaat aaagtgacta aaaattaaac aaataccctt 3420
taagaaatta aaaaaactaa ggaaacattt ttcttgtttc gagtagataa tgccagcctg 3480
ttaaacgccg tcgacgagtc taacggacac caaccagcga accagcagcg tcgcgtcggg 3540
ccaagcgaag cagacggcac ggcatctctg tcgctgcctc tggacccctc tcgagagttc 3600
cgctccaccg ttggacttgc tccgctgtcg gcatccagaa attgcgtggc ggagcggcag 3660
acgtgagccg gcacggcagg cggcctcctc ctcctctcac ggcacggcag ctacgggggа 3720
ttcctttccc accgctcctt cgctttccct tcctcgcccg ccgtaataaa tagacacccc 3780
ctccacaccc tctttcccca acctcgtgtt gttcggagcg cacacacaca           3830
```


Copy provided by USPTO from the PACR Image Database on 07/20/2004

<210> 27
<211> 3347
<212> DNA
<213> Artificial Sequence

<220>
<223> Sequence that represents part of the PHI8999A insert as well as flanking sequence 3' to the insert.

<400> 27
cccactatcc ttcgcaagac ccttcctcta tataaggaag ttcatttcat ttggagagga 60
cagggtaccc ggggatccac catgtctccg gagaggagac cagttgagat taggccagct 120
acagcagctg atatggccgc ggtttgtgat atcgttaacc attacattga gacgtctaca 180
gtgaacttta ggacagagcc acaaacacca caagagtgga ttgatgatct agagaggttg 240
caagatagat acccttggtt ggttgctgag gttgagggtg ttgtggctgg tattgcttac 300
gctgggccct ggaaggctag gaacgcttac gattggacag ttgagagtac tgtttacgtg 360
tcacataggc atcaaaggtt gggcctagga tccacattgt acacacattt gcttaagtct 420
atggaggcgc aaggttttaa gtctgtggtt gctgttatag gccttccaaa cgatccatct 480
gttaggttgc atgaggcttt gggatacaca gcccggggta cattgcgcgc agctggatac 540
aagcatggtg gatggcatga tgttggtttt tggcaaaggg attttgagtt gccagctcct 600
ccaaggccag ttaggccagt tacccagatc tgagtcgacc tgcaggcatg cccgctgaaa 660
tcaccagtct ctctctacaa atctatctct ctctataata atgtgtgagt agttcccaga 720
taagggaatt agggttctta tagggtttcg ctcatgtgtt gagcatataa gaaaccctta 780
gtatgtattt gtatttgtaa aatacttcta tcaataaaat ttctaattcc taaaaccaaa 840
atccagtggc gagctcgaat tcgagctcga gcccgggtgg atcctctaga gtcgacctgc 900
agaagcttcg gtccggcgcg cctctagttg aagacacgtt catgtcttca tcgtaagaag 960
acactcagta gtcttcggcc agaatggcct aactcaaggc cctcactccg cttgatcttg 1020
gcaaagatat ttgacgcatt tattagtatg tgttaatttt catttgcagt gcagtatttt 1080
ctattcgatc tttatgtaat tcgttacaat taataaatat tcaaatcaga ttattgactg 1140
tcatttgtat caaatcgtgt ttaatggata tttttattat aatattgatg atatctcaat 1200
caaaacgtag ataataataa tatttattta atatttttgc gtcgcacagt gaaaatctat 1260
atgagattac aaaataccga caacattatt taagaaacat agacattaac cctgagactg 1320
ttggacatca acgggtagat tccttcatgc atagcacctc attcttgggg acaaaagcac 1380
ggtttggccg ttccattgct gcacgaacga gctttgctat atcctcgggt tggatcatct 1440
catcaggtcc aatcaaattt gtccaagaac tcatgttagt cgcaacgaaa ccggggcata 1500
tgtcgggtat ctcgagctcg cgaaagcttg gctgcaggtc gacggatcct tcaacaaaag 1560
ggtacctgta cccgaaaccg acacaggtgg gtaggtagag aatacctagg ggcgcgagac 1620
aactctctct aaggaactcg gcaaaatagc cccgtaactt cgggagaagg ggtgcccccc 1680
gctaacaata aacgaatacg gtttatgtat ggattccggt aaaataccgg tactcgattt 1740
cataagagtc gaataggaag ttaagatgag ggtggtatca tcataaaaat ggagtagtat 1800
cctaaattat actaatccac gtatgatatg tatgcctttc cttatcaacc ggaagtagtg 1860
caaaaaaaat tctatactgc actgctctct ttttactgag aaatgcaaaa aaataaaagt 1920
gaagtaaggg tgccccatag atatttgatc ttgcctcctg tccccccccc ccttttttca 1980
tcaaaaattt ccatgaaaaa agaaaagatg aatttgtcca ttcattgaac cctagttcgg 2040
gactgacggg gctcgaaccc gcagcttccg cctgttccta gccttccagg gcccagcgta 2100
agcaatacca gccacagcac cctcaacctc agcaaccaac caagggtatc tatcttgcaa 2160
cctctctaga tcatcaatcc actcttgtgg tgtttgtggc tctgtcctaa agttcactgt 2220
agacgtctca atgtaatggt taacgatatc acaaaccgcg gaacacaaga acgaaagcac 2280
cttttcattc tttcatatac taggggtttt tacttggaaa agacaatgtt ccatactaaa 2340
ggatagctgc agaagccgcc accgtcttga ggaccttccg gggagccaga ccggtcgaac 2400
cgtgcctcca cttgctaagg agaaagggaa aatcagggcc aggacatacg aaggaggagc 2460
cagaacgaag atatcctaag atacttactc gctccgggcc atgatcaatc atgcctgtgg 2520
ggaggtctct cgcacctcga tccatgaagg taccaccgag gtctgccccg ccgccggctt 2580
cggtaccgtc ctcgccttgg gcgcccgagg cacccggggg atggactgcc caggcgcagc 2640
cacgacgacc caaggatcac cctcctgcgc agtcggcacg agcaatagtt ctcggggaac 2700
aggcagcttg gcctgactcc ccggggtcac ctcaactacc tcggccgagg ggtcaagtac 2760
cccctcagtc cgcccccgct cttcggaccg ggaccccgac gtcccggccc cggataccga 2820

Copy provided by USPTO from the PACR Image Database on 07/20/2004

cggcaccagc ccgctcgggg gctggcttga cgacccctgg cccagcctca gatctgggct 2880
gaggccgagg caggcggcca tgtcgtcgtc ttcatcatcg tcttcatcat cgtcgtcgtc 2940
atcaggcgtc tccggcgacg gctcccttgg gagcccctcc ctctcctgcc gacgacgaag 3000
cctttccaag gcatcccgag cccacgtccg ctcgtgggcc cgagccttct ttgcgtcctt 3060
cttctccttc ctcttctccg cggtgaccct ccgcgcagct cggtccaccg catcctccgg 3120
gactggtggc agggaaggct tgtgatgccc tacctcctgg agacagacga aaagtctcag 3180
ctatgagaac cgagggcaat ctgacgcaag aaggaagaag gagcggatac tcaccagaga 3240
cacgcacccg cgatcgggac gcattaaggg ctgggaaaaa gtgccggcct ctaatttcgc 3300
taccgtgccg tccacccacc tgtggaggtc atcgatggga aggggaa 3347

<210> 28
<211> 669
<212> DNA
<213> Zea mays

<400> 28
actagtttcc tagcccgcgt cgtgccccta ccccaccgac gtttatggaa ggtgccattc 60
cacggttctt cgtggccgcc cctaaggatg taaatggtcg gtaaaatccg gtaaatttcc 120
ggtaccgttt accagatttt tccagccgtt ttcggattta tcgggatata cagaaaacga 180
gacggaaacg gaataggttt tttttcgaaa acggtacggt aaacggtgag acaaacttac 240
cgtccgtttt cgtatttctc gggaaactct ggtatattcc cgtatttgtc ccgtattttc 300
ccgacccacg gacctgccaa tcaaccatca gccagtcagc ccatccccac agctatggcc 360
catggggcca tgttggccac atgcccacgc aacgcaaggc agtaaggctg gcagcctggc 420
acgcattgac gcatgtggac acacacagcc gccgcctgtt cgtgtttctg tgccgttgtg 480
cgagactgtg actgcgagtg gcggagtcgg cgaacggcga ggcgtctccg gagtctggac 540
tgcggctgtg gacagcgacg ctgtgacggc gactcggcga agccccaagc taccaagccc 600
ccaagtcccc atccatctct gcttctctgg tcatctcctt cccctggtcg atctgcaggc 660
gccagaccg 669

<210> 29
<211> 200
<212> DNA
<213> Zea mays

<400> 29
gccgaagcat cacgaaacgc actaagacct cgaaggagtc aaaccactcc tccgaggcct 60
cgggggctac acccggcggg tgcgctcgcg cgcacccacc ggaacaaaat gtaaccgaga 120
aaggtcggtc cccttgcaaa aaaagtgcga caaaagcctc caagcgagta ttaacactca 180
ctttgaggct cgggggctac 200

<210> 30
<211> 812
<212> DNA
<213> Zea mays

<220>
<223> Fragment of maize Huck-1 retrotransposon

<400> 30
tgtcggggac cataattagg ggtaccccca agactcctaa tctcagctgg taacccccat 60
cagcacaaag ctgcaaaggc ctgatgggtg cgattaagtc aaggctcggt ccactcaagg 120
gacacgatct cgcctcgccc gagcccagcc tcgggcaagg gcggccgacc ccgaggattc 180
acgtctcgcc cgagggcccc ctcaagcgac gggcacacct tcggctcgcc cgaggcccat 240
tcttcgccga gaagcaacct tggccagatc gccacaccga ccgaccgtat cgcaggagca 300
tttaatgcga ggatcgcctg acaccttatc ctgacgcgcg ctcttcagtc gacagagccg 360
aagtgaccgc aatcacttcg ccgctccact gaccgacctg acaagaagac agcgccgcct 420
gcgtcgctcc gactgctgtg ccactcgaca gagtgaggct gacagcagcc aagtccggcc 480
tcgggcgcca taggaagctc cgcctcgccc gaccctaggg ctcggactcg gcctcggctc 540

Copy provided by USPTO from the PACR Image Database on 07/20/2004

cggaagacga cgaactacgc ttcgcccgac cccagggctt ggactcagcc tcggctccgg 600
aagacgacga attccgcctc gcccgacccc agggctcgga ctcggcctcg gctccagaag 660
acgacgaact ccgcctcgcc cgaccccagg gctcggactc agcctcggct ccggaagacg 720
acgaactccg cctcgcccga ccccagggct cggactcagc ctcggcctca gacgatggtc 780
tccgcctcgc ccgacccggg gctcggactc ga 812

<210> 31
<211> 335
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert sequence -
fragment of cry1F gene

<400> 31
cctttctatc ggaccttgtc agatcctgtc ttcgtccgag gaggctttgg caatcctcac 60
tatgtactcg gtcttagggg agtggccttt caacaaactg gtacgaatca cacccgcaca 120
ttcaggaact ccgggaccat tgactctcta gatgagatac cacctcaaga caacagcggc 180
gcaccttgga atgactactc ccatgtgctg aatcatgtta cctttgtgcg ctggccaggt 240
gagatctcag gttccgactc atggagagca ccaatgttct cttggacgca tcgtagcgct 300
acccccacaa acaccattga tccagagaga atcac 335

<210> 32
<211> 321
<212> DNA
<213> Zea mays

<220>
<223> Fragment of maize chloroplast rpoC2 gene

<400> 32
tcattcttca agaactgcat atcttgccga gatcctcatc cctaaaggta cttgacaata 60
gtattattgg agtcgataca caactcacaa aaaatacaag aagtcgacta ggtggattgg 120
tccgagtgaa gagaaaaaaa agccatacag aactcaaaat cttttccgga gatattcatt 180
ttcctgaaga ggcggataag atattaggtg gcagtttgat accaccagaa agagaaaaaa 240
aagattctaa ggaatcaaaa aaaaggaaaa attgggttta tgttcaacgg aaaaaatttc 300
tcaaaagcaa ggaaaagtat t 321

<210> 33
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert
sequence-fragment of ubiZM1(2) promoter; also a
fragment of the maize chloroplast trn1 gene

<400> 33
gtggctattt atctatc 17

<210> 34
<211> 201
<212> DNA
<213> Artificial Sequence

<220>

Copy provided by USPTO from the PACR Image Database on 07/26/2004

<223> Represents part of PHI8999A insert sequence -
fragment of pat gene

<400> 34
gcagctgata tggccgcggt ttgtgatatc gttaaccatt acattgagac gtctacagtg 60
aactttagga cagagccaca aacaccacaa gagtggattg atgatctaga gaggttgcaa 120
gatagatacc cttggttggt tgctgaggtt gagggtgttg tggctggtat tgcttacgct 180
gggccctgga aggctaggaa c 201

<210> 35
<211> 138
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert sequence -
fragment of pat gene (complement)

<400> 35
cctcaacctc agcaaccaac caatggtatc tatcttgcaa cctctctaga tcatcaatcc 60
actcttgtgg tgtttgtggc tctgtcctaa agttcactgt agacgtctca atgtaatggt 120
taacgatatc acaaaccg 138

<210> 36
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert sequence -
fragment of cry1F gene (complement)

<400> 36
agagaagagg gatct 15

<210> 37
<211> 118
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert sequence -
fragment of polylinker

<400> 37
cgaagcttcg gccggggccc atcgatatcc gcgggcatgc ctgcagtgca gcgtgacccg 60
gtcgtgcccc tctctagaga taatgagcat tgcatgtcta agttataaaa aattacca 118

<210> 38
<211> 550
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert sequence -
fragment of ORF25 terminator (complement)

<400> 38

Copy provided by USPTO from the PACR Image Database on 07/20/2004

ctcactccgc ttgatcttgg caaagatatt tgacgcattt attagtatgt gttaattttc 60
atttgcagtg cagtattttc tattcgatct ttatgtaatt cgttacaatt aataaatatt 120
caaatcagat tattgactgt catttgtatc aaatcgtgtt taatggatat ttttattata 180
atattgatga tatctcaatc aaaacgtaga taataataat atttatttaa tatttttgcg 240
tcgcacagtg aaaatctata tgagattaca aaataccgac aacattattt aagaaacata 300
gacattaacc ctgagactgt tggacatcaa cgggtagatt ccttcatgca tagcacctca 360
ttcttgggga caaaagcacg gtttggccgt tccattgctg cacgaacgag ctttgctata 420
tcctcgggtt ggatcatctc atcaggtcca atcaaatttg tccaagaact catgttagtc 480
gcaacgaaac cggggcatat gtcgggtatc tcgagctcgc gaaagcttgg ctgcaggtcg 540
acggatcctt 550

<210> 39
<211> 128
<212> DNA
<213> Zea mays

<220>
<223> Fragment of maize chloroplast rps12 rRNA gene
(complement)

<400> 39
caacaaaagg gtacctgtac ccgaaaccga cacaggtggg taggtagaga atacctaggg 60
gcgcgagaca actctctcta aggaactcgg caaaatagcc ccgtaacttc gggagaaggg 120
gtgccccc 128

<210> 40
<211> 392
<212> DNA
<213> Zea mays

<220>
<223> Fragment of maize chloroplast genome

<400> 40
ctaacaataa acgaatacgg tttatgtatg gattccggta aaataccggt actcgatttc 60
ataagagtcg aataggaagt taagatgagg gtggtatcat cataaaaatg gagtagtatc 120
ctaaattata ctaatccacg tatgatatgt atgcctttcc ttatcaaccg gaagtagtgc 180
aaaaaaaatt ctatactgca ctgctctctt tttactgaga aatgcaaaaa aataaaagtg 240
aagtaagggt gccccataga tatttgatct tgcctcctgt ccccccccc ctttttttcat 300
caaaaatttc catgaaaaaa gaaaagatga atttgtccat tcattgaacc ctagttcggg 360
actgacgggg ctcgaacccg cagcttccgc ct 392

<210> 41
<211> 188
<212> DNA
<213> Artificial Sequence

<220>
<223> Represents part of PHI8999A insert sequence -
fragment of pat gene (complement)

<400> 41
gttcctagcc ttccagggcc cagcgtaagc aataccagcc acagcaccct caacctcagc 60
aaccaaccaa gggtatctat cttgcaacct ctctagatca tcaatccact cttgtggtgt 120
ttgtggctct gtcctaaagt tcactgtaga cgtctcaatg taatggttaa cgatatcaca 180
aaccgcgg 188

<210> 42

Copy provided by USPTO from the PACR Image Database on 07/20/2004

<211> 81
<212> DNA
<213> Zea mays

<220>
<223> Fragment of maize chloroplast ORF241 (complement)

<400> 42
cacaagaacg aaagcacctt ttcattcttt catatactag gggtttttac ttggaaaaga 60
caatgttcca tactaaagga t 81

<210> 43
<211> 254
<212> DNA
<213> Zea mays

<400> 43
agctgcagaa gccgccaccg tcttgaggac cttccgggga gccagaccgg tcgaaccgtg 60
cctccacttg ctaaggagaa agggaaaatc agggccagga catacgaagg aggagccaga 120
acgaagatat cctaagatac ttactcgctc cgggccatga tcaatcatgc ctgtggggag 180
gtctctcgca cctcgatcca tgaaggtacc accgaggtct gccccgccgc cggcttcggt 240
accgtcctcg cctt 254

<210> 44
<211> 749
<212> DNA
<213> Zea mays

<400> 44
gggcgcccga ggcacccggg ggatggactg cccaggcgca gccacgacga cccaaggatc 60
accctcctgc gcagtcggca cgagcaatag ttctcgggga acaggcagct tggcctgact 120
ccccggggtc acctcaacta cctcggccga ggggtcaagt acccccctcag tccgcccccg 180
ctcttcggac cgggaccccg acgtcccggc cccggatacc gacggcacca gcccgctcgg 240
gggctggctt gacgacccct ggcccagcct cagatctggg ctgaggccga ggcaggcggc 300
catgtcgtcg tcttcatcat cgtcttcatc atcgtcgtcg tcatcaggcg tctccggcga 360
cggctccctt gggagcccct ccctctcctg ccgacgacga agcctttcca aggcatcccg 420
agcccacgtc cgctcgtggg cccgagcctt ctttgcgtcc ttcttctcct tcctcttctc 480
cgcggtgacc ctccgcgcag ctcggtccac cgcatcctcc gggactggtg gcagggaagg 540
cttgtgatgc cctacctcct ggagacagac gaaaagtctc agctatgaga accgagggca 600
atctgacgca agaaggaaga aggagcggat actcaccaga gacacgcacc cgcgatcggg 660
acgcattaag ggctgggaaa aagtgccggc ctctaatttc gctaccgtgc cgtccaccca 720
cctgtggagg tcatcgatgg gaaggggaa 749

<210> 45
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between
regions 3 and 4

<400> 45
tcggactcga cctttctatc 20

<210> 46
<211> 20
<212> DNA

Copy provided by USPTO from the PACR Image Database on 07/26/2004

<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 4 and 5

<400> 46
agagaatcac tcattcttca 20

<210> 47
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 5 and 6

<400> 47
gaaaagtatt gtggctattt 20

<210> 48
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 6 and 7a

<400> 48
atctatccgt gcagctgata 20

<210> 49
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 7a and 7b

<400> 49
ggctaggaac cctcaacctc 20

<210> 50
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 7b and 7c

<400> 50
tcacaaaccg agagaagagg 20

<210> 51

Copy provided by USPTO from the PACR Image Database on 07/20/2004

<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 7c and 8

<400> 51
agagggatct cgaagcttcg 20

<210> 52
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 5' event flanking sequence; junction between regions 8 and 9

<400> 52
aaaattacca caactggaag 20

<210> 53
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' event flanking sequence; junction between regions 9 and 10

<400> 53
agctatgttt ctcactccgc 20

<210> 54
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' event flanking sequence; junction between regions 10 and 11

<400> 54
acggatcctt caacaaaagg 20

<210> 55
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' event flanking sequence; junction between regions 11 and 12

<400> 55
gtgccccccg ctaacaataa 20

Copy provided by USPTO from the PACR Image Database on 07/20/2004

<210> 56
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' event flanking sequence; junction between
regions 12 and 13

<400> 56
gcttccgcct gttcctagcc 20

<210> 57
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> 3' event flanking sequence; junction between
regions 13 and 14

<400> 57
caaaccgcgg cacaagaacg 20

Copy provided by USPTO from the PACR Image Database on 07/28/2004

Europäisches Patentamt
European Patent Office
Office européen des brevets

Request for grant of a European patent

For official use only		
1 Application number:	MKEY	**MKEY - EP15168051.9**
2 Date of receipt (Rule 35(2) EPC):	DREC	**DREC - 18 May 2015**
3 Date of receipt at EPO (Rule 35(4) EPC):	RENA	
4 Date of filing:		

5 Grant of European patent, and examination of the application under Article 94, are hereby requested. ☒

5.1 The applicant waives his right to be asked whether he wishes to proceed further with the application (Rule 70(2)) ☐

Procedural language: en

Description and/or claims filed in: en

6 Applicant's or representative's reference: N96314A-EP

Applicant 1

7-1 Name: Dow AgroSciences, LLC

8-1 Address: 9330 Zionsville Road
Indianapolis IN Indiana 46268
United States of America

10-1 State of residence or of principal place of business: United States of America

Applicant 2

7-2 Name: E.I. Du Pont de Nemours and Company

8-2 Address: 1007 Market Street
Wilmington DE Delaware 19898
United States of America

10-1 State of residence or of principal place of business: United States of America

Applicant 3

7-3 Name: PIONEER HI-BRED INTERNATIONAL, INC.

8-3 Address: 7100 N.W. 62nd Avenue
Johnston IA Iowa 50131-1014
United States of America

14.1 The/Each applicant hereby declares that he is an entity or a natural person under Rule 6(4) EPC. ☐

Representative 1

15-1 Name: BENTHAM, Mr Andrew

Company: J A Kemp

16-1 Address of place of business: 14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

17-1 Telephone: +44 20 3077 8600

17-1 Fax: +44 20 7242 8932

17-1 E-mail: mail@jakemp.com

Inventor(s)

23 Inventor details filed separately ☒

24 **Title of invention**

Title of invention: CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

25 **Declaration of priority (Rule 52)**

A declaration of priority is hereby made for the following applications

	State	Filing date	Kind	Application number:	Search results under Rule 141(1) are attached
Priority 01	US	02.05.2003	pr	60/467772	☐

25.2 This application is a complete translation of the previous application ☐

25.3 It is not intended to file a (further) declaration of priority ☐

26 **Reference to a previously filed application**

27 **Divisional application** ☒

Application number of earlier application: EP04751097.9

Date of Filing (Art. 80/Rule 40 EPC): 29 April 2004

27.1 This divisional application is of the following generation: ☒ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 or subsequent

28 **Article 61(1)(b) application** ☐

29 **Claims**

Number of claims: 15

29.1 ☒ as attached

29.2 ☐ as in the previously filed application (see Section 26.2)

29.3 ☐ The claims will be filed later

30 **Figures**

It is proposed that the abstract be published together with figure No.

31 **Designation of contracting states**

All the contracting states party to the EPC and valid for the parent application at the time of filing of this divisional application are deemed to be designated (see Article 76(2)).

32 **Different applicants for different contracting states**

33 **Extension/Validation**

This application is deemed to be a request to extend the effects of the European patent application and the European patent granted in respect of it to all non-contracting states to the EPC with which extension or validation agreements are in force on the date on which the application is filed. However, the request is deemed withdrawn if the extension fee or the validation fee, whichever is applicable, is not paid within the prescribed time limit.

33.1 It is intended to pay the extension fee(s) for the following state(s):

33.2 It is intended to pay the validation fee(s) for the following state(s):

34 **Biological material**

38 **Nucleotide and amino acid sequences**

The European patent application contains a sequence listing as part of the description ☒

The sequence listing is attached in computer-readable format in accordance with WIPO Standard ST.25 ☒

The sequence listing is attached in PDF format ☐

Further indications

39 Additional copies of the documents cited in the European search report are requested

Number of additional sets of copies:

40 Refund of the search fee under to Article 9 of the Rules relating to Fees is requested ☒

Application or publication number of earlier search report: 04751097.9

42 Payment

Mode of payment: Debit from deposit account

The European Patent Office is hereby authorised, to debit from the deposit account with the EPO any fees and costs indicated on the fees section below.

Currency: EUR

Deposit account number: 28050038

Account holder: J A Kemp

43 Refunds

Fees	Factor applied	Fee schedule	Amount to be paid
001 Filing fee - EP direct - online	1	120.00	120.00
002 Fee for a European search - Applications filed on/after 01.07.2005	1	1 285.00	1 285.00
015 Claims fee - For the 16th to the 50th claim	0	235.00	0.00
015e Claims fee - For the 51st and each subsequent claim	0	580.00	0.00
033 Renewal fee for the 3rd year	1	465.00	465.00
034 Renewal fee for the 4th year	1	580.00	580.00
035 Renewal fee for the 5th year	1	810.00	810.00
036 Renewal fee for the 6th year	1	1 040.00	1 040.00
037 Renewal fee for the 7th year	1	1 155.00	1 155.00
038 Renewal fee for the 8th year	1	1 265.00	1 265.00
039 Renewal fee for the 9th year	1	1 380.00	1 380.00
040 Renewal tee for the 10th year	1	1 560.00	1 560.00
041 Renewal fee for the 11th year	1	1 560.00	1 560.00
042 Renewal fee for the 12th year	1	1 560.00	1 560.00
501 Additional filing fee for the 36th and each subsequent page	27	15.00	405.00
Total:		**EUR**	**13 185.00**

44-A Forms

	Details:	System file name:
A-1	Request	as ep-request.pdf

A-2	1. Designation of inventor	1. Inventor	as f1002-1.pdf

44-B Technical documents

		Original file name:	System file name:
B-1	Specification	N96314A-EP AXB KKT mqr Spec as filed - May15.pdf Description; 15 claims; abstract	SPECEPO-1.pdf
B-2	Specification	N96314A-EP AXB KKT mqr Figure filed - May15.pdf 1 figure(s)	SPECEPO-2.pdf
B-3	Sequence listings, ASCII	N96314A-EP AXB KKT Sequence Listing - May15.txt	SEQLTXT.txt

44-C Other documents

		Original file name:	System file name:
C-1	Track change claims	N96314A-EP AXB KKT mqr Track change claims - May15.pdf	OTHER-1.pdf
C-2	Inernational Search Report on Parent Application	N96314-EP AXB KKT mqr Search Report.pdf	OTHER-2.pdf
C-3	Covering letter	N96314A-EP AXB KKT mqr File new Div App - May15.pdf	OTHER-3.pdf

45 General authorisation:

46 Signature(s)

Place:

Date: **18 May 2015**

Signed by: **Andrew Bentham 23464**

Representative name: **Andrew BENTHAM**

Capacity: **(Representative)**

J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

18 May 2015

Dear Sirs

New Divisional European Patent Application
Based on European Patent Application No. 04751097.9
DOW AGROSCIENCES LLC *et al*.
Our Ref: N96314A-EP AXB/KKT/mqr

We are hereby filing a new Divisional application. We attach the following:

1 A Request Form.

2 A Designation of Inventor.

3 An Application text consisting of the complete Description, Claims, Sequence Listing, Abstract and Figures (the Sequence Listing being filed as a .txt file).

4 A copy of the International Search Report issued by the EPO on the parent Application in the International phase (PCT Application No. PCT/US2004/013538/European Application No. 04751097.9).

5. A debit order drawn on J A Kemp's EPO deposit account number 2805.0038 for payment of the filing fee (EUR 120), search fee (EUR 1285), page fees for every page beyond the thirty-fifth (EUR 15 each, totalling EUR 405), and the renewal fee for years three to twelve (EUR 11,375). Please deduct the amount of any underpayment in the fees from J A Kemp's deposit account number 2805.0038.

6. In order to assist the Examiner in assessing the basis for the claims of the Divisional, an amended copy of the original claims of the parental PCT Application No. PCT/US2004/013538, showing in track-changes how the claims of the Divisional are arrived at, is attached.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7242 8932
mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

1 Amendments & Basis

The Application text for the Divisional application is identical to that of the Parent Application as filed except that (a) we have amended the claims and (b) inserted a list of numbered items of the Invention at the end of description (see pages 45 to 52). The numbered items correspond to the claims of the original PCT application, but do not at present form claims for the Divisional application.

For the purpose of Article 76(1) EPC, basis in the Parent Application for the claims of this new Divisional Application is set out in the Table below

New Claims	Basis in European Application No. 04751097.9 as originally filed
1	Page 1 lines 8 to 9 and 13 to 14 ("*insect resistant corn plant TC1507*"); Page 7 line 10 ("*TC1507 corn plant or parts thereof*"); Page 13 lines 5 to 6 and 12 ("*parts thereof*" and "*corn seed*")
2	Page 1 lines 8 to 9 and 13 to 14 ("*insect resistant corn plant TC1507*"); Page 7 line 10 ("*TC1507 corn plant or parts thereof*"); Page 7 lines 19 to 20 and Figure 1 ("*SEQ ID NO: 24*"); Page 13 lines 5 to 6 and 12 ("*parts thereof*" and "*corn seed*"); Page 6 to 10 of the Sequence listing as filed ("*SEQ ID NO: 24*"); Page 30 to 31 (Table 1) ("*SEQ ID NO: 24*")
3	Page 6 lines 12 o 18; and Page 12 line 28 to page 13, line 4
4	Original claims 51 to 53
5	Original claim 54
6	Original claims 1 to 5
7	Original claims 6 to 14
8	Original claims 15, 16, 19 and 62 to 64
9	Original claims 17 to 18
10	Original claims 23 to 38, 43, 49, 50 and 59 to 61
11	Original claims 39 to 42
12	Original claims 44, 45, 48 and 55
13	Original claims 46, 47, 56 and 57
14	Original claim 65
15	Original claim 66

2 Other Matters

The subject matter covered by the claims of the Divisional application is similar to that of part of the original claims of the Parent application which was the subject of the International Search Report. Accordingly, we request a refund of the search fee.

The EPO is requested to place a copy of the US priority applications filed on the parent case on the file of this Divisional application.

The Sequence Listing now filed as a txt file forms part of the application as filed.

I confirm that, to the best of our knowledge and belief, the Sequence Listing now filed does not include matter which goes beyond the content of the Parent application as filed. The Sequence Listing is the same as that originally filed on the parental application.

Yours faithfully

Electronically Signed
A BENTHAM

INTERNATIONAL SEARCH REPORT

ational Application No
../US2004/013538

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, MEDLINE, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANONYMOUS: "DAS-01507-1 (TC1507)" INTERNET ARTICLE, 'Online! 17 October 2002 (2002-10-17), XP002312770 Retrieved from the Internet: URL:http://www.agbios.com/dbase.php?action =showprod&data=TC1507&frmat=long> 'retrieved on 2005-01-04! the whole document -----	1-22,43, 49-54, 59-66
X	ANONYMOUS: "Skov- og Naturstyrelsen" INTERNET ARTICLE, 'Online! 11 December 2000 (2000-12-11), XP002312769 DENMARK Retrieved from the Internet: URL:http://www.sns.dk/erhvogadm/hoeringer/ c_nl_00_10/indhold.htm> 'retrieved on 2005-01-06! the whole document -/--	1-43,46, 47, 49-54, 56-66

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 January 2005	22/02/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Helliot, B

Form PCT/ISA/210 (second sheet) (January 2004)

INTERNATIONAL SEARCH REPORT

ational Application No
.../US2004/013538

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& Summary notification information format for the placing on the market (import) of 1507 maize in accordance with directive 2001/18/EC. (February, 14th 2003), in "Deliberate releases and placing on the EU market of Genetically Modified Organisms (GMOs)". http://gmoinfo.jrc.it/gmc_browse.asp the whole document	
X	US 2002/162136 A1 (HOHN THOMAS M ET AL) 31 October 2002 (2002-10-31) sequence 1	44,45,55
X	DATABASE EMBL 'Online! 18 November 2002 (2002-11-18), "Mus musculus chromosome 5 clone rp23-426e16 strain C57BL/6J, complete sequence." XP002312771 retrieved from EBI Database accession no. AC084071 the whole document	48,55

INTERNATIONAL SEARCH REPORT

ational Application No
.../US2004/013538

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2002162136 A1	31-10-2002	US 6346655 B1	12-02-2002
		US 2004034884 A1	19-02-2004

Form PCT/ISA/210 (patent family annex) (January 2004)

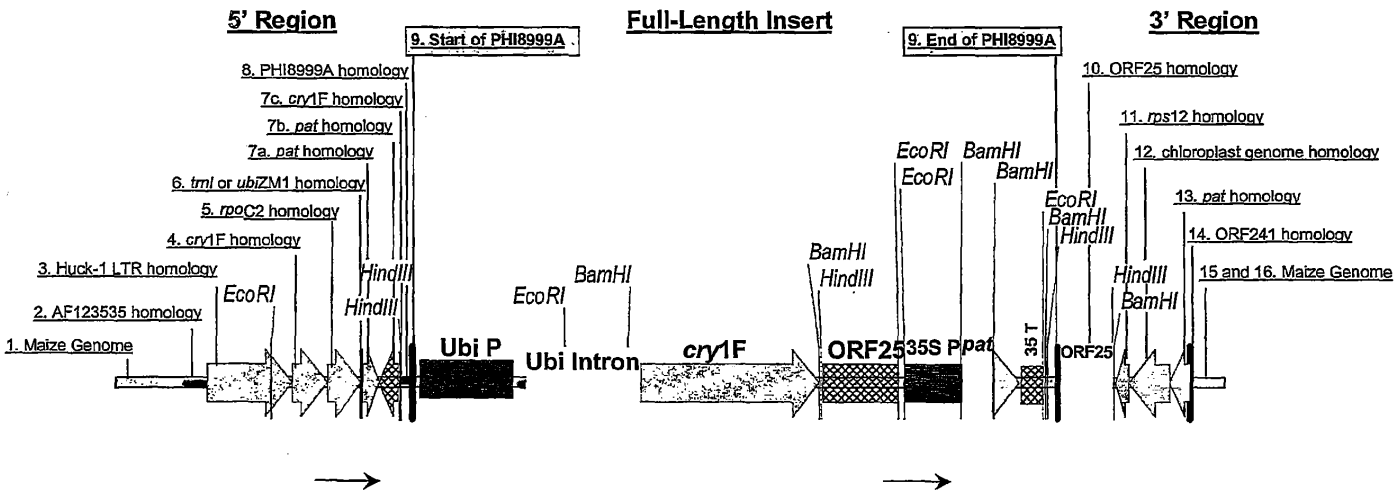

5' Region
Full-Length Insert
3' Region
9. Start of PHI8999A
9. End of PHI8999A
8. PHI8999A homology
7c. cry1F homology
7b. pat homology
7a. pat homology
6. trnI or ubiZM1 homology
5. rpoC2 homology
4. cry1F homology
3. Huck-1 LTR homology
2. AF123535 homology
1. Maize Genome
EcoRI
HindIII
HindIII
EcoRI
BamHI
Ubi P
Ubi Intron
cry1F
BamHI
HindIII
EcoRI
EcoRI
BamHI
BamHI
EcoRI
BamHI
HindIII
ORF25
35S P
pat
35 T
ORF25
10. ORF25 homology
11. rps12 homology
12. chloroplast genome homology
13. pat homology
14. ORF241 homology
15 and 16. Maize Genome
HindIII
BamHI

FIG. 1

Form 1002 - 1: Public inventor(s)

Designation of inventor

User reference: N96314A-EP
Application No:

Public

	Inventor	
	Name:	BING James W.
	Address:	1565 N.E. 118th Avenue Ankeny IA Iowa 50021 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	BARBOUR Eric
	Address:	6075 Annapolis Lanc N. Plymouth MN Minnesota 55446 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	CARDINEAU Guy A.
	Address:	18 West Cottage Lane Tempe AZ Arizona 85282 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	CRESSMAN Robert F., Jr.
	Address:	2502 Turnstone Drive Wilmington DE Delaware 19805 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	GUPTA Manju
	Address:	13463 Winamac Court Carmel IN Indiana 46032 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:

	Inventor	
	Name:	HARTNETT LOCKE Mary E.
	Address:	31 Millwood Drive Mickleton NJ New Jersey 08056 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	HONDRED David
	Address:	1432 N.W. 72nd Avenue Ankeny IA Iowa 50021 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	KEASCHALL Joseph W.
	Address:	13563 Summit Drive Clive IA Iowa 50325 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	KOZIEL Michael G.
	Address:	1601 Liatris Lane Raleigh NC North Carolina 27613 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	MEYER Terry E.
	Address:	4338 101st Street Urbandale IA Iowa 50322 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	MOELLENBECK Daniel
	Address:	11655 N.W. 107th Court Granger IA Iowa 50109 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:

	Inventor	
	Name:	NARVA Kenneth E.
	Address:	2856 Via Conquistador Carlsbad CA California 92009 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	NIRUNSUKSIRI Wilas
	Address:	515 Aaby Drive Auburn WA Washington 98001 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	RITCHIE Steven W.
	Address:	16218 Wright Circle Omaha NE Nebraska 68130 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	RUDERT Marjorie L.
	Address:	1364 274th Lane Boone IA Iowa 50036 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	SANDERS Craig D.
	Address:	15 Penny Lane Bear DE Delaware 19701 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	SHAO Aihua
	Address:	501 Malinmor Drive St Charles MO Missouri 63304 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:

	Inventor	
	Name:	STELMAN Steven J.
	Address:	11221 Via Madonna Santee CA California 92071 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	STUCKER David S.
	Address:	9028 Timberwood Drive Johnston IA Iowa 50131 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	TAGLIANI Laura A.
	Address:	4100 Huntsman Drive Zionsville IN Indiana 46077 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:
	Inventor	
	Name:	VAN ZANTE William M.
	Address:	4424 73rd Place Urbandale IA Iowa 50322 United States of America
	The applicant has acquired the right to the European patent:	Under agreement:

CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

FIELD OF THE INVENTION

The present invention relates to the field of plant molecular biology, specifically the invention relates to a DNA construct for conferring insect resistance to a plant. The invention more specifically relates to an insect resistant corn plant TC1507 and to assays for detecting the presence of corn plant TC1507 DNA in a sample and compositions thereof.

BACKGROUND OF THE INVENTION

This invention relates to the insect resistant corn (*Zea mays*) plant TC1507, also referred to as maize line TC1507 or maize event TC1507, and to the DNA plant expression construct of corn plant TC1507 and the detection of the transgene/flanking insertion region in corn plant TC1507 and progeny thereof.

Corn is an important crop and is a primary food source in many areas of the world. Damage caused by insect pests is a major factor in the loss of the world's corn crops, despite the use of protective measures such as chemical pesticides. In view of this, insect resistance has been genetically engineered into crops such as corn in order to control insect damage and to reduce the need for traditional chemical pesticides. One group of genes which have been utilized for the production of transgenic insect resistant crops are the delta-endotoxins from *Bacillus thuringiensis (B.t.)*. Delta-endotoxins have been successfully expressed in crop plants such as cotton, potatoes, rice, sunflower, as well as corn, and have proven to provide excellent control over insect pests. (Perlak, F.J *et al.* (1990) *Bio/Technology* 8, 939-943; Perlak, F.J. *et al.* (1993) *Plant Mol. Biol.* 22: 313-321; Fujimoto H. *et al.* (1993) *Bio/Technology* 11: 1151-1155; Tu *et al.* (2000) *Nature Biotechnology* 18:1101-1104; PCT publication number WO 01/13731; and Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, Fort Collins, CO).

The expression of foreign genes in plants is known to be influenced by their location in the plant genome, perhaps due to chromatin structure (e.g., heterochromatin) or the proximity of transcriptional regulatory elements (e.g., enhancers) close to the integration site (Weising *et al.*, *Ann. Rev. Genet* 22:421-477, 1988). At the same time the presence of the transgene at different locations in the genome will influence the overall

phenotype of the plant in different ways. For this reason, it is often necessary to screen a large number of events in order to identify an event characterized by optimal expression of an introduced gene of interest. For example, it has been observed in plants and in other organisms that there may be a wide variation in levels of expression of an introduced gene among events. There may also be differences in spatial or temporal patterns of expression, for example, differences in the relative expression of a transgene in various plant tissues, that may not correspond to the patterns expected from transcriptional regulatory elements present in the introduced gene construct. For this reason, it is common to produce hundreds to thousands of different events and screen those events for a single event that has desired transgene expression levels and patterns for commercial purposes. An event that has desired levels or patterns of transgene expression is useful for introgressing the transgene into other genetic backgrounds by sexual outcrossing using conventional breeding methods. Progeny of such crosses maintain the transgene expression characteristics of the original transformant. This strategy is used to ensure reliable gene expression in a number of varieties that are well adapted to local growing conditions.

It would be advantageous to be able to detect the presence of a particular event in order to determine whether progeny of a sexual cross contain a transgene of interest. In addition, a method for detecting a particular event would be helpful for complying with regulations requiring the pre-market approval and labeling of foods derived from recombinant crop plants, for example, or for use in environmental monitoring, monitoring traits in crops in the field, or monitoring products derived from a crop harvest, as well as for use in ensuring compliance of parties subject to regulatory or contractual terms.

It is possible to detect the presence of a transgene by any nucleic acid detection method known in the art including, but not limited to, the polymerase chain reaction (PCR) or DNA hybridization using nucleic acid probes. These detection methods generally focus on frequently used genetic elements, such as promoters, terminators, marker genes, etc., because for many DNA constructs, the coding region is interchangeable. As a result, such methods may not be useful for discriminating between different events, particularly those produced using the same DNA construct or very similar constructs unless the DNA sequence of the flanking DNA adjacent to the inserted heterologous DNA is known. For example, an event-specific PCR assay is described in U.S. Patent No. 6,395,485 for the detection of elite event GAT-ZM1. Accordingly, it would be desirable to have a simple and discriminative method for the identification of event TC1507.

SUMMARY OF THE INVENTION

This invention relates preferably to methods for producing and selecting an insect resistant monocot crop plant. More specifically, a DNA construct is provided that when expressed in plant cells and plants confers resistance to insects. According to one aspect of the invention, a DNA construct, capable of introduction into and replication in a host cell, is provided that when expressed in plant cells and plants confers insect resistance to the plant cells and plants. The DNA construct is comprised of a DNA molecule named PHI8999A and it includes two transgene expression cassettes. The first expression cassette comprises a DNA molecule which includes the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689 and Christensen and Quail (1996) *Transgenic Res.* 5:213-218) operably connected to a DNA molecule encoding a *B.t.* δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350). The second transgene expression cassette of the DNA construct comprises a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59) operably connected to a DNA molecule encoding a phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene* 70: 25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59). Plants containing the DNA construct are also provided.

According to another aspect of the invention, compositions and methods are provided for identifying a novel corn plant designated TC1507, which methods are based on primers or probes which specifically recognize the 5' and/or 3' flanking sequence of TC1507. DNA molecules are provided that comprise primer sequences that when utilized in a PCR reaction will produce amplicons unique to the transgenic event TC1507. These molecules may be selected from the group consisting of:

5'-GTAGTACTATAGATTATATTATTCGTAGAG-3' (SEQ ID NO: 1);

5'-GCCATACAGAACTCAAAATCTTTTCCGGAG-3' (SEQ ID NO: 2);

5'- CTTCAAACAAGTGTGACAAA –3' (SEQ ID NO: 23);

5'-TGTGGTGTTTGTGGCTCTGTCCTAA-3' (SEQ ID NO: 3);

5'-AGCACCTTTTCATTCTTTCATATAC-3' (SEQ ID NO: 4);

5'-GACCTCCCCA CAGGCATGAT TGATC-3' (SEQ ID NO: 5);

and complements thereof. The corn plant and seed comprising these molecules is an aspect of this invention. Further, kits utilizing these primer sequences for the identification of the TC1507 event are provided.

An additional aspect of the invention relates to the specific flanking sequences of TC1507 described herein, which can be used to develop specific identification methods for TC1507 in biological samples. More particularly, the invention relates to the 5' and/or 3' flanking regions of TC1507, SEQ ID NO:21 and SEQ ID NO:22, respectively, which can be used for the development of specific primers and probes. The invention further relates to identification methods for the presence of TC1507 in biological samples based on the use of such specific primers or probes.

According to another aspect of the invention, methods of detecting the presence of DNA corresponding to the corn event TC1507 in a sample are provided. Such methods comprise: (a) contacting the sample comprising DNA with a DNA primer set, that when used in a nucleic acid amplification reaction with genomic DNA extracted from corn event TC1507 produces an amplicon that is diagnostic for corn event TC1507; (b) performing a nucleic acid amplification reaction, thereby producing the amplicon; and (c) detecting the amplicon.

DNA molecules that comprise the novel transgene/flanking insertion region, SEQ ID NO: 26 and SEQ ID NO: 27 and are homologous or complementary to SEQ ID NO: 26 and SEQ ID NO: 27 are an aspect of this invention.

DNA sequences that comprise the novel transgene/flanking insertion region, SEQ ID NO:26 are an aspect of this invention. DNA sequences that comprise a sufficient length of polynucleotides of transgene insert sequence and a sufficient length of polynucleotides of maize genomic and/or flanking sequence from maize plant TC1507 of SEQ ID NO:26 that are useful as primer sequences for the production of an amplicon product diagnostic for maize plant TC1507 are included.

In addition, DNA sequences that comprise the novel transgene/flanking insertion region, SEQ ID NO:27 are provided. DNA sequences that comprise a sufficient length of polynucleotides of transgene insert sequence and a sufficient length of polynucleotides of maize genomic and/or flanking sequence from maize plant TC1507 of SEQ ID NO:27 that are useful as primer sequences for the production of an amplicon product diagnostic for maize plant TC1507 are included.

According to another aspect of the invention, the DNA sequences that comprise at least 11 or more nucleotides of the transgene portion of the DNA sequence of SEQ ID

NO:26 or complements thereof, and a similar length of 5' flanking maize DNA sequence of SEQ ID NO:26 or complements thereof are useful as DNA primers in DNA amplification methods. The amplicons produced using these primers are diagnostic for maize event TC1507. Therefore, the invention also includes the amplicons produced by DNA primers homologous or complementary to SEQ ID NO:26.

According to another aspect of the invention, the DNA sequences that comprise at least 11 or more nucleotides of the transgene portion of the DNA sequence of SEQ ID NO:27 or complements thereof, and a similar length of 3' flanking maize DNA sequence of SEQ ID NO:27 or complements thereof are useful as DNA primers in DNA amplification methods. The amplicons produced using these primers are diagnostic for maize event TC1507. Therefore, the invention also includes the amplicons produced by DNA primers homologous or complementary to SEQ ID NO:27.

More specifically, a pair of DNA molecules comprising a DNA primer set, wherein the DNA molecules are identified as SEQ ID NO: 1 or complements thereof and SEQ ID NO: 2 or complements thereof; SEQ ID NO: 2 or complements thereof and SEQ ID NO: 23 or complements thereof; SEQ ID NO: 3 or complements thereof and SEQ ID NO: 5 or complements thereof; SEQ ID NO: 4 or complements thereof and SEQ ID NO: 5 or complements thereof are aspects of the invention.

Further aspects of the invention include the amplicon comprising the DNA molecules of SEQ ID NO: 1 and SEQ ID NO: 2; the amplicon comprising the DNA molecules of SEQ ID NO: 2 and SEQ ID NO: 23; the amplicon comprising the DNA molecules of SEQ ID NO: 3 and SEQ ID NO: 5; and the amplicon comprising the DNA molecules of SEQ ID NO: 4 and SEQ ID NO: 5.

According to another aspect of the invention, methods of detecting the presence of a DNA molecule corresponding to the TC1507 event in a sample, such methods comprising: (a) contacting the sample comprising DNA extracted from a corn plant with a DNA probe, molecule that hybridizes under stringent hybridization conditions with DNA extracted from corn event TC1507 and does not hybridize under the stringent hybridization conditions with a control corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA. More specifically, a method for detecting the presence of a DNA molecule corresponding to the TC1507 event in a sample, such methods, consisting of (a) contacting the sample comprising DNA extracted from a corn plant with a DNA probe molecule that consists of sequences that are unique to the event, e.g. junction sequences, wherein said DNA probe

molecule hybridizes under stringent hybridization conditions with DNA extracted from corn event TC1507 and does not hybridize under the stringent hybridization conditions with a control corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA.

In addition, a kit and methods for identifying event TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24 are provided.

DNA molecules are provided that comprise at least one junction sequence of TC1507 selected from the group consisting of SEQ ID NO:45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; wherein a junction sequence spans the junction between heterologous DNA inserted into the genome and the DNA from the corn cell flanking the insertion site, i.e. flanking DNA, and is diagnostic for the TC1507 event.

According to another aspect of the invention, methods of producing an insect resistant corn plant that comprise the steps of: (a) sexually crossing a first parental corn line comprising the expression cassettes of the present invention, which confers resistance to insects, and a second parental corn line that lacks insect resistance, thereby producing a plurality of progeny plants; and (b) selecting a progeny plant that is insect resistant. Such methods may optionally comprise the further step of back-crossing the progeny plant to the second parental corn line to producing a true-breeding corn plant that is insect resistant.

The present invention provides a method of producing a corn plant that is resistant to insects comprising transforming a corn cell with the DNA construct PHI8999A (SEQ ID NO:25), growing the transformed corn cell into a corn plant, selecting the corn plant that shows resistance to insects, and further growing the corn plant into a fertile corn plant. The fertile corn plant can be self pollinated or crossed with compatible corn varieties to produce insect resistant progeny.

The invention further relates to a DNA detection kit for identifying maize event TC1507 in biological samples. Preferably the kit of the invention comprises a first primer which specifically recognizes the 5' or 3' flanking region of TC1507, and a second primer which specifically recognizes a sequence within the foreign DNA of TC1507, or within the flanking DNA, for use in a PCR identification protocol. The invention also relates to a kit for identifying event TC1507 in biological samples, which kit comprises a specific probe having a sequence which corresponds or is complementary to, a sequence having between 80% and 100% sequence identity with a specific region of event TC1507. Preferably the sequence of the probe corresponds to a specific region comprising part of the 5' or 3' flanking region of event TC1507.

The methods and kits encompassed by the present invention can be used for different purposes such as, but not limited to the following: to identify event TC1507 in plants, plant material or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material; additionally or alternatively, the methods and kits of the present invention can be used to identify transgenic plant material for purposes of segregation between transgenic and non-transgenic material; additionally or alternatively, the methods and kits of the present invention can be used to determine the quality of plant material comprising maize event TC1507. The kits may also contain the reagents and materials necessary for the performance of the detection method.

This invention further relates to the TC1507 corn plant or its parts, including, but not limited to, pollen, ovules, vegetative cells, the nuclei of pollen cells, and the nuclei of egg cells of the corn plant TC1507 and the progeny derived thereof. The corn plant and seed TC1507 from which the DNA primer molecules of the present invention provide a specific amplicon product is an aspect of the invention.

The foregoing and other aspects of the invention will become more apparent from the following detailed description and accompanying drawing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Linear map showing the transgenic insert PHI8999A, as well as the sequences flanking the transgenic insert.

DETAILED DESCRIPTION

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al.*, *Glossary of Genetics: Classical and Molecular, 5th edition,* Springer-Verlag; New York, 1991; and Lewin, *Genes V,* Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at 37 CFR 1.822 is used.

As used herein, the term "comprising" means "including but not limited to".

As used herein, the term "corn" means *Zea mays* or maize and includes all plant varieties that can be bred with corn, including wild maize species.

As used herein, the term "TC1507 specific" refers to a nucleotide sequence which is suitable for discriminatively identifying event TC1507 in plants, plant material, or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material.

As used herein, the terms "insect resistant" and "impacting insect pests" refers to effecting changes in insect feeding, growth, and/or behavior at any stage of development, including but not limited to: killing the insect; retarding growth; preventing reproductive capability; and the like.

As used herein, the terms "pesticidal activity" and "insecticidal activity" are used synonymously to refer to activity of an organism or a substance (such as, for example, a protein) that can be measured by numerous parameters including, but not limited to, pest mortality, pest weight loss, pest attraction, pest repellency, and other behavioral and physical changes of a pest after feeding on and/or exposure to the organism or substance for an appropriate length of time. For example "pesticidal proteins" are proteins that display pesticidal activity by themselves or in combination with other proteins.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. As used herein, the terms "encoding" or "encoded" when used in the context of a specified nucleic acid mean that the nucleic acid comprises the requisite information to guide translation of the nucleotide sequence into a specified protein. The information by which a protein is encoded is specified by the use of codons. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid or may lack such intervening non-translated sequences (e.g., as in cDNA).

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. "Foreign" refers to material not normally found in the location of interest. Thus "foreign DNA" may comprise both recombinant DNA as well as newly introduced,

rearranged DNA of the plant. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The site in the plant genome where a recombinant DNA has been inserted may be referred to as the "insertion site" or "target site".

As used herein, "insert DNA" refers to the heterologous DNA within the expression cassettes used to transform the plant material while "flanking DNA" can exist of either genomic DNA naturally present in an organism such as a plant, or foreign (heterologous) DNA introduced via the transformation process which is extraneous to the original insert DNA molecule, e.g. fragments associated with the transformation event. A "flanking region" or "flanking sequence" as used herein refers to a sequence of at least 20 base pair, preferably at least 50 base pair, and up to 5000 base pair which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the original foreign insert DNA molecule. Transformation procedures leading to random integration of the foreign DNA will result in transformants containing different flanking regions characteristic and unique for each transformant. When recombinant DNA is introduced into a plant through traditional crossing, its flanking regions will generally not be changed. Transformants will also contain unique junctions between a piece of heterologous insert DNA and genomic DNA, or 2 pieces of genomic DNA, or 2 pieces of heterologous DNA. A "junction" is a point where 2 specific DNA fragments join. For example, a junction exists where insert DNA joins flanking DNA. A junction point also exists in a transformed organism where 2 DNA fragments join together in a manner that is modified from that found in the native organism. "Junction DNA" refers to DNA that comprises a junction point.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous nucleotide sequence can be from a species different from that from which the nucleotide sequence was derived, or, if from the same species, the promoter is not naturally found operably linked to the nucleotide sequence. A heterologous protein may originate from a foreign species, or, if from the same species, is substantially modified from its original form by deliberate human intervention.

"Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements are often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters that cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect numerous parameters including, processing of the primary transcript to mRNA, mRNA stability and/or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene

expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht *et al.* (1989) *Plant Cell* 1:671-680.

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide.

A DNA construct is an assembly of DNA molecules linked together that provide one or more expression cassettes. The DNA construct may be a plasmid that is enabled for self replication in a bacterial cell and contains various endonuclease enzyme restriction sites that are useful for introducing DNA molecules that provide functional genetic elements, i.e., promoters, introns, leaders, coding sequences, 3' termination regions, among others; or a DNA construct may be a linear assembly of DNA molecules, such as an expression cassette. The expression cassette contained within a DNA construct comprise the necessary genetic elements to provide transcription of a messenger RNA. The expression cassette can be designed to express in prokaryote cells or eukaryotic cells. Expression cassettes of the present invention are designed to express most preferably in plant cells.

The DNA molecules of the invention are provided in expression cassettes for expression in an organism of interest. The cassette will include 5' and 3' regulatory sequences operably linked to a coding sequence of the invention. "Operably linked" means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. Operably linked is intended to indicate a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes or multiple DNA constructs.

The expression cassette will include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region, a coding region, and a transcriptional and translational termination region functional in the organism serving as a host. The transcriptional initiation region (*i.e.*, the promoter) may be native or analogous, or foreign or heterologous to the host organism. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. The expression cassettes may additionally

contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation.

It is to be understood that as used herein the term "transgenic" includes any cell, cell line, callus, tissue, plant part, or plant the genotype of which has been altered by the presence of a heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

A transgenic "event" is produced by transformation of plant cells with a heterologous DNA construct(s), including a nucleic acid expression cassette that comprises a transgene of interest, the regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and selection of a particular plant characterized by insertion into a particular genome location. An event is characterized phenotypically by the expression of the transgene. At the genetic level, an event is part of the genetic makeup of a plant. The term "event" also refers to progeny produced by a sexual outcross between the transformant and another variety that include the heterologous DNA. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed parent is present in the progeny of the cross at the same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA and flanking sequence immediately adjacent to the inserted DNA that would be expected to be transferred to a progeny that receives inserted DNA including the transgene of interest as the result of a sexual cross of one parental line that includes the inserted DNA (e.g., the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA.

An insect resistant TC1507 corn plant can be bred by first sexually crossing a first parental corn plant consisting of a corn plant grown from the transgenic TC1507 corn plant and progeny thereof derived from transformation with the expression cassettes of the present invention that confers insect resistance, and a second parental corn plant that lacks insect resistance, thereby producing a plurality of first progeny plants; and then selecting a first progeny plant that is resistant to insects; and selfing the first progeny plant, thereby producing a plurality of second progeny plants; and then selecting from the second

progeny plants an insect resistant plant. These steps can further include the back-crossing of the first insect resistant progeny plant or the second insect resistant progeny plant to the second parental corn plant or a third parental corn plant, thereby producing a corn plant that is resistant to insects.

As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, and progeny of same. Parts of transgenic plants understood to be within the scope of the invention comprise, for example, plant cells, protoplasts, tissues, callus, embryos as well as flowers, stems, fruits, leaves, and roots originating in transgenic plants or their progeny previously transformed with a DNA molecule of the invention and therefore consisting at least in part of transgenic cells, are also an aspect of the present invention.

As used herein, the term "plant cell" includes, without limitation, seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants that can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere *et al.* (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or "gene gun" transformation technology (Klein *et al.* (1987) *Nature* (London) 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Additional transformation methods are disclosed below.

Thus, isolated polynucleotides of the present invention can be incorporated into recombinant constructs, typically DNA constructs, which are capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels *et al.*, (1985; Supp. 1987) *Cloning Vectors: A Laboratory Manual*, Weissbach and Weissbach (1989) *Methods for Plant Molecular Biology*, (Academic Press, New York); and Flevin *et al.*, (1990) *Plant Molecular Biology Manual*, (Kluwer Academic Publishers). Typically, plant expression vectors include, for example, one or more cloned

plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several references, e.g., Fehr, in *Breeding Methods for Cultivar Development*, Wilcos J. ed., American Society of Agronomy, Madison Wis. (1987).

A "probe" is an isolated nucleic acid to which is attached a conventional detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, or enzyme. Such a probe is complementary to a strand of a target nucleic acid, in the case of the present invention, to a strand of isolated DNA from corn event TC1507 whether from a corn plant or from a sample that includes DNA from the event. Probes according to the present invention include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that bind specifically to a target DNA sequence and can be used to detect the presence of that target DNA sequence.

"Primers" are isolated nucleic acids that are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase. Primer pairs of the present invention refer to their use for amplification of a target nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods. "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (see, U.S. Patent Nos. 4,683,195 and 4,800,159; herein incorporated by reference).

Probes and primers are of sufficient nucleotide length to bind to the target DNA sequence specifically in the hybridization conditions or reaction conditions determined by the operator. This length may be of any length that is of sufficient length to be useful in a detection method of choice. Generally, 11 nucleotides or more in length, preferably 18

nucleotides or more, and more preferably 22 nucleotides or more, are used. Such probes and primers hybridize specifically to a target sequence under high stringency hybridization conditions. Preferably, probes and primers according to the present invention have complete DNA sequence similarity of contiguous nucleotides with the target sequence, although probes differing from the target DNA sequence and that retain the ability to hybridize to target DNA sequences may be designed by conventional methods. Probes can be used as primers, but are generally designed to bind to the target DNA or RNA and not be used in an amplification process.

Specific primers can be used to amplify an integration fragment to produce an amplicon that can be used as a "specific probe" for identifying event TC1507 in biological samples. When the probe is hybridized with the nucleic acids of a biological sample under conditions which allow for the binding of the probe to the sample, this binding can be detected and thus allow for an indication of the presence of event TC1507 in the biological sample. Such identification of a bound probe has been described in the art. The specific probe is preferably a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the event and preferably also comprises a part of the foreign DNA contiguous therewith. Preferably the specific probe comprises a sequence of at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, and most preferably between 95 and 100% identical (or complementary) to a specific region of the event.

Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual, 2nd ed.*, vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*, Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992"); and Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as the PCR primer analysis tool in Vector NTI version 6 (Informax Inc., Bethesda MD); PrimerSelect (DNASTAR Inc., Madison, WI); and Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Additionally, the sequence can be visually scanned and primers manually identified using guidelines known to one of skill in the art.

A "kit" as used herein refers to a set of reagents for the purpose of performing the method of the invention, more particularly, the identification of the event TC1507 in biological samples. The kit of the invention can be used, and its components can be specifically adjusted, for purposes of quality control (e.g. purity of seed lots), detection of event TC1507 in plant material, or material comprising or derived from plant material, such as but not limited to food or feed products. "Plant material" as used herein refers to material which is obtained or derived from a plant.

Primers and probes based on the flanking DNA and insert sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed sequences by conventional methods, e.g., by re-cloning and sequencing such sequences. The nucleic acid probes and primers of the present invention hybridize under stringent conditions to a target DNA sequence. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of DNA from a transgenic event in a sample. Nucleic acid molecules or fragments thereof are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, 1989, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, a Practical Approach,* IRL Press, Washington, D.C. (1985), departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

In hybridization reactions, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. The thermal melting point (Tm) is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. For DNA-DNA hybrids, the Tm can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: Tm = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. Tm is reduced by about 1°C for each 1% of mismatching; thus, Tm, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the Tm can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the Tm for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the Tm; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the Tm; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the Tm.

Using the equation, hybridization and wash compositions, and desired Tm, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a Tm of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

As used herein, a substantially homologous sequence is a nucleic acid molecule that will specifically hybridize to the complement of the nucleic acid molecule to which it is being compared under high stringency conditions. Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2X SSC at 50°C, are known to those skilled in the art

or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of a destabilizing agent such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. In a preferred embodiment, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules unique to the TC1507 event or complements thereof or fragments of either under moderately stringent conditions.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0); the ALIGN PLUS program (version 3.0, copyright 1997); and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 10 (available from Accelrys, 9685 Scranton Road, San Diego, CA 92121, USA). Alignments using these programs can be performed using the default parameters.

The CLUSTAL program is well described by Higgins and Sharp, *Gene* 73: 237-244 (1988); Higgins and Sharp, *CABIOS* 5: 151-153 (1989); Corpet, *et al., Nucleic Acids Research* 16: 10881-90 (1988); Huang, *et al., Computer Applications in the Biosciences* 8: 155-65 (1992), and Pearson, *et al., Methods in Molecular Biology* 24: 307-331 (1994). The ALIGN and the ALIGN PLUS programs are based on the algorithm of Myers and Miller (1988) *supra.* The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra.* The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology,* Chapter 19, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). Alignment may also be performed manually by inspection.

To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) supra. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.hlm.nih.gov.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a

conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Regarding the amplification of a target nucleic acid sequence (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize only to the target nucleic-acid sequence to which a primer having the corresponding wild-type sequence (or its complement) would bind and preferably to produce a unique amplification product, the amplicon, in a DNA thermal amplification reaction.

The term "specific for (a target sequence)" indicates that a probe or primer hybridizes under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

As used herein, "amplified DNA" or "amplicon" refers to the product of nucleic acid amplification of a target nucleic acid sequence that is part of a nucleic acid template. For example, to determine whether a corn plant resulting from a sexual cross contains transgenic event genomic DNA from the corn plant of the present invention, DNA extracted from the corn plant tissue sample may be subjected to a nucleic acid amplification method using a DNA primer pair that includes a first primer derived from flanking sequence adjacent to the insertion site of inserted heterologous DNA, and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the event DNA. Alternatively, the second primer may be

derived from the flanking sequence. The amplicon is of a length and has a sequence that is also diagnostic for the event. The amplicon may range in length from the combined length of the primer pairs plus one nucleotide base pair to any length of amplicon producible by a DNA amplification protocol. Alternatively, primer pairs can be derived from flanking sequence on both sides of the inserted DNA so as to produce an amplicon that includes the entire insert nucleotide sequence of the PHI8999A expression construct, see FIG. 1, approximately 6.2 Kb in size. A member of a primer pair derived from the flanking sequence may be located a distance from the inserted DNA sequence, this distance can range from one nucleotide base pair up to the limits of the amplification reaction, or about twenty thousand nucleotide base pairs. The use of the term "amplicon" specifically excludes primer dimers that may be formed in the DNA thermal amplification reaction.

Nucleic acid amplification can be accomplished by any of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described, inter alia, in U.S. Pat. Nos. 4,683,195 and 4,683,202 and in *PCR Protocols: A Guide to Methods and Applications,* ed. Innis *et al.,* Academic press, San Diego, 1990. PCR amplification methods have been developed to amplify up to 22 Kb of genomic DNA and up to 42 Kb of bacteriophage DNA (Cheng *et al., Proc. Natl. Acad. Sci. USA* 91:5695-5699, 1994). These methods as well as other methods known in the art of DNA amplification may be used in the practice of the present invention. It is understood that a number of parameters in a specific PCR protocol may need to be adjusted to specific laboratory conditions and may be slightly modified and yet allow for the collection of similar results. These adjustments will be apparent to a person skilled in the art.

The amplicon produced by these methods may be detected by a plurality of techniques, including, but not limited to, Genetic Bit Analysis (Nikiforov, *et al. Nucleic Acid Res.* 22:4167-4175, 1994) where a DNA oligonucleotide is designed which overlaps both the adjacent flanking DNA sequence and the inserted DNA sequence. The oligonucleotide is immobilized in wells of a microwell plate. Following PCR of the region of interest (using one primer in the inserted sequence and one in the adjacent flanking sequence) a single-stranded PCR product can be hybridized to the immobilized oligonucleotide and serve as a template for a single base extension reaction using a DNA polymerase and labeled ddNTPs specific for the expected next base. Readout may be fluorescent or ELISA-based. A signal indicates presence of the insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Another detection method is the Pyrosequencing technique as described by Winge (*Innov. Pharma. Tech.* 00: 18-24, 2000). In this method an oligonucleotide is designed that overlaps the adjacent DNA and insert DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted sequence and one in the flanking sequence) and incubated in the presence of a DNA polymerase, ATP, sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. dNTPs are added individually and the incorporation results in a light signal which is measured. A light signal indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single or multi-base extension.

Fluorescence Polarization as described by Chen *et al.*, (*Genome Res.* 9:492-498, 1999) is a method that can be used to detect an amplicon of the present invention. Using this method an oligonucleotide is designed which overlaps the flanking and inserted DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted DNA and one in the flanking DNA sequence) and incubated in the presence of a DNA polymerase and a fluorescent-labeled ddNTP. Single base extension results in incorporation of the ddNTP. Incorporation can be measured as a change in polarization using a fluorometer. A change in polarization indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Taqman® (PE Applied Biosystems, Foster City, Calif.) is described as a method of detecting and quantifying the presence of a DNA sequence and is fully understood in the instructions provided by the manufacturer. Briefly, a FRET oligonucleotide probe is designed which overlaps the flanking and insert DNA junction. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Hybridization of the FRET probe results in cleavage and release of the fluorescent moiety away from the quenching moiety on the FRET probe. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

Molecular Beacons have been described for use in sequence detection as described in Tyangi *et al.* (*Nature Biotech.* 14:303-308, 1996). Briefly, a FRET oligonucleotide probe is designed that overlaps the flanking and insert DNA junction. The unique structure of the FRET probe results in it containing secondary structure that keeps the

fluorescent and quenching moieties in close proximity. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Following successful PCR amplification, hybridization of the FRET probe to the target sequence results in the removal of the probe secondary structure and spatial separation of the fluorescent and quenching moieties. A fluorescent signal results. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

A hybridization reaction using a probe specific to a sequence found within the amplicon is yet another method used to detect the amplicon produced by a PCR reaction.

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLES

Example 1. Transformation of Maize by Particle Bombardment and Regeneration of Transgenic Plants Containing the Cry1F Gene

A DNA molecule of 6.2 Kb, designated PHI8999A (see FIG. 1 and SEQ ID NO:25), which includes a first transgene expression cassette comprising the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) Plant Mol. Biol. 18:675-689 and Christensen and Quail (1996) Transgenic Res. 5:213-218) operably connected to a DNA molecule encoding a Bacillus thuringiensis δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350), and a second transgene expression cassette comprising a DNA molecule of the cauliflower

mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) operably connected to a DNA molecule encoding the selectable marker, phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene 70*:25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) was used to transform maize embryo tissue.

B.t. Cry1F maize plants were obtained by microprojectile bombardment using the Biolistics® PDS-1000He particle gun manufactured by Bio-Rad, Hercules, CA; essentially as described by Klein *et al.* (1987) *Nature,* UK 327(6117):70-73. Immature embryos isolated from maize ears, harvested soon after pollination were cultured on callus initiation medium for several days. On the day of transformation, microscopic tungsten particles were coated with purified PHI8999A DNA (SEQ ID NO:25) and accelerated into the cultured embryos, where the insert DNA was incorporated into the cell chromosome. Only insert PHI8999A was used during transformation and no additional plasmid DNA was incorporated into the transformant. After bombardment, embryos were transferred to callus initiation medium containing glufosinate as the selection agent. Individual embryos were kept physically separate during culture, and the majority of explants died on the selective medium.

Those embryos that survived and produced healthy, glufosinate-resistant callus tissue were assigned unique identification codes representing putative transformation events, and continually transferred to fresh selection medium. Plants were regenerated from tissue derived from each unique event and transferred to the greenhouse. Leaf samples were taken for molecular analysis to verify the presence of the transgene by PCR and to confirm expression of the Cry1F protein by ELISA. Plants were then subjected to a whole plant bioassay using European corn borer insects. Positive plants were crossed with inbred lines to obtain seed from the initial transformed plants. A number of lines were evaluated in the field. The TC1507 event was selected from a population of independent transgenic events based on a superior combination of characteristics, including insect resistance and agronomic performance (see Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, fort Collins, CO, herein incorporated by reference).

Example 2. Identification of nucleotides comprising the flanking sequence 5' to the transgenic insert DNA in *Bacillus thuringiensis* Cry1F maize line TC1507

To identify a DNA fragment that included sequence 5' to the PHI8999A insert in event TC1507, Spe I restriction enzyme fragments from event TC1507 genomic DNA were size selected on agarose gels, purified, and screened by Southern analysis to confirm hybridization to a Cry1F probe. Following confirmation of hybridization and fragment size, the fragments of interest were cloned into a pBluescript II SK (+)™ cloning vector to prepare an enriched size selected plasmid based genomic DNA library. A probe homologous to a portion of the Cry1F gene was used to screen the plasmid library for positive clones. A positive clone was identified, purified by additional screening, and confirmed to result in a positive signal when hybridized to the Cry1F probe. Nearly 3 Kb of the Spe I fragment contained in the isolated positive clone was sequenced using a primer walking approach. To initiate the first sequencing run, a primer that binds to a known sequence in the cloning vector DNA was designed to sequence a portion of the DNA of interest. A second sequencing run over the same region using another primer oriented in the reverse direction provided second strand coverage. Primer walking was accomplished by repeatedly using sequence data from previous runs to design new primers that were then used to extend the next round of sequencing further into the DNA of interest until the flanking sequence 5' to the inserted transgenic DNA in maize event TC1507 was obtained. Specific sequence information is provided in Example 4.

Example 3. Confirmation of flanking sequence 5' to the *B.t.* Cry1F maize line TC1507 insert

To confirm the 5' flanking sequence of the *B.t.* Cry1F maize line TC1507 insert, PCR primer pairs were designed to obtain overlapping PCR products extending from the 5' flanking region into the full-length PHI8999A transgenic insert. PCR products were successfully amplified from *B.t.* Cry1F maize line TC1507 genomic DNA, isolated, and sequenced for Region 1 through Region 6, shown in Table 1, and confirmed to match the previously determined sequence from the Spe I fragment, described in Example 2. However, the region from bp 2358 to bp 2829, immediately adjacent and 5' to the start of the full-length insert was recalcitrant to PCR amplification and appeared to be larger than the sequence obtained from the Spe I clone described above. The use of primer pairs flanking this region and the Advantage®-GC 2 Polymerase Mix (BD Biosciences Clontech, Palo Alto, CA) was successful in amplifying PCR products from *B.t.* Cry1F maize line

TC1507 genomic DNA for sequencing. The amplification conditions used to produce amplicons with the Advantage®-GC 2 system are shown in Table 10. The DNA primer pairs used to confirm the sequence in the region from bp 2358 to 2829 are those listed in SEQ ID NO:1 and SEQ ID NO:2; and SEQ ID NO:2 and SEQ ID NO:23. Sequence from this region is described in Table 1 (Regions 7a, 7b, 7c, and 8).

Example 4. Event TC1507 5' flanking sequence. A description of each region is provided in Table 1.

Region 1 (SEQ ID NO: 28) Maize genomic (no significant homology)

1 ACTAGTTTCC TAGCCCGCGT CGTGCCCCTA CCCCACCGAC
GTTTATGGAA
51 GGTGCCATTC CACGGTTCTT CGTGGCCGCC CCTAAGGATG
TAAATGGTCG
101 GTAAAATCCG GTAAATTTCC GGTACCGTTT ACCAGATTTT
TCCAGCCGTT
151 TTCGGATTTA TCGGGATATA CAGAAAACGA GACGGAAACG
GAATAGGTTT
201 TTTTTCGAAA ACGGTACGGT AAACGGTGAG ACAAACTTAC
CGTCCGTTTT
251 CGTATTTCTC GGGAAACTCT GGTATATTCC CGTATTTGTC
CCGTATTTTC
301 CCGACCCACG GACCTGCCAA TCAACCATCA GCCAGTCAGC
CCATCCCCAC
351 AGCTATGGCC CATGGGGCCA TGTTGGCCAC ATGCCCACGC
AACGCAAGGC
401 AGTAAGGCTG GCAGCCTGGC ACGCATTGAC GCATGTGGAC
ACACACAGCC
451 GCCGCCTGTT CGTGTTTCTG TGCCGTTGTG CGAGACTGTG
ACTGCGAGTG
501 GCGGAGTCGG CGAACGGCGA GGCGTCTCCG GAGTCTGGAC
TGCGGCTGTG
551 GACAGCGACG CTGTGACGGC GACTCGGCGA AGCCCCAAGC
TACCAAGCCC
601 CCAAGTCCCC ATCCATCTCT GCTTCTCTGG TCATCTCCTT
CCCCTGGTCG
651 ATCTGCAGGC GCCAGACCG

Region 2 (SEQ ID NO: 29) Undescribed maize genomic sequence (complement)

670 G CCGAAGCATC ACGAAACGCA CTAAGACCTC
701 GAAGGAGTCA AACCACTCCT CCGAGGCCTC GGGGGCTACA
CCCGGCGGGT
751 GCGCTCGCGC GCACCCACCG GAACAAAATG TAACCGAGAA
AGGTCGGTCC
801 CCTTGCAAAA AAAGTGCGAC AAAAGCCTCC AAGCGAGTAT
TAACACTCAC
851 TTTGAGGCTC GGGGGCTAC

Region 3 (SEQ ID NO: 30) Fragment of maize Huck-1 retrotransposon

870 T GTCGGGGACC ATAATTAGGG GTACCCCCAA
901 GACTCCTAAT CTCAGCTGGT AACCCCCATC AGCACAAAGC TGCAAAGGCC
951 TGATGGGTGC GATTAAGTCA AGGCTCGGTC CACTCAAGGG ACACGATCTC
1001 GCCTCGCCCG AGCCCAGCCT CGGGCAAGGG CGGCCGACCC CGAGGATTCA
1051 CGTCTCGCCC GAGGGCCCCC TCAAGCGACG GGCACACCTT CGGCTCGCCC
1101 GAGGCCCATT CTTCGCCGAG AAGCAACCTT GGCCAGATCG CCACACCGAC
1151 CGACCGTATC GCAGGAGCAT TTAATGCGAG GATCGCCTGA CACCTTATCC
1201 TGACGCGCGC TCTTCAGTCG ACAGAGCCGA AGTGACCGCA ATCACTTCGC
1251 CGCTCCACTG ACCGACCTGA CAAGAAGACA GCGCCGCCTG CGTCGCTCCG
1301 ACTGCTGTGC CACTCGACAG AGTGAGGCTG ACAGCAGCCA AGTCCGGCCT
1351 CGGGCGCCAT AGGAAGCTCC GCCTCGCCCG ACCCTAGGGC TCGGACTCGG
1401 CCTCGGCTCC GGAAGACGAC GAACTACGCT TCGCCCGACC CCAGGGCTTG
1451 GACTCAGCCT CGGCTCCGGA AGACGACGAA TTCCGCCTCG CCCGACCCCA
1501 GGGCTCGGAC TCGGCCTCGG CTCCAGAAGA CGACGAACTC CGCCTCGCCC
1551 GACCCCAGGG CTCGGACTCA GCCTCGGCTC CGGAAGACGA CGAACTCCGC
1601 CTCGCCCGAC CCCAGGGCTC GGACTCAGCC TCGGCCTCAG ACGATGGTCT
1651 CCGCCTCGCC CGACCCGGGG CTCGGACTCG A

Region 4 (SEQ ID NO: 31) Fragment of *cry*1F gene

1682 CCTTTCTAT CGGACCTTGT
1701 CAGATCCTGT CTTCGTCCGA GGAGGCTTTG GCAATCCTCA CTATGTACTC
1751 GGTCTTAGGG GAGTGGCCTT TCAACAAACT GGTACGAATC ACACCCGCAC
1801 ATTCAGGAAC TCCGGGACCA TTGACTCTCT AGATGAGATA CCACCTCAAG
1851 ACAACAGCGG CGCACCTTGG AATGACTACT CCCATGTGCT GAATCATGTT
1901 ACCTTTGTGC GCTGGCCAGG TGAGATCTCA GGTTCCGACT CATGGAGAGC
1951 ACCAATGTTC TCTTGGACGC ATCGTAGCGC TACCCCCACA AACACCATTG

2001 ATCCAGAGAG AATCAC

Region 5 (SEQ ID NO: 32) Fragment of maize chloroplast *rpo*C2 gene

2017 TCAT TCTTCAAGAA CTGCATATCT TGCCGAGATC
2051 CTCATCCCTA AAGGTACTTG ACAATAGTAT TATTGGAGTC
GATACACAAC
2101 TCACAAAAAA TACAAGAAGT CGACTAGGTG GATTGGTCCG
AGTGAAGAGA
2151 AAAAAAAGCC ATACAGAACT CAAAATCTTT TCCGGAGATA
TTCATTTTCC
2201 TGAAGAGGCG GATAAGATAT TAGGTGGCAG TTTGATACCA
CCAGAAAGAG
2251 AAAAAAAAGA TTCTAAGGAA TCAAAAAAAA GGAAAAATTG
GGTTTATGTT
2301 CAACGGAAAA AATTTCTCAA AAGCAAGGAA AAGTATT

Region 6 (SEQ ID NO: 33) Fragment of maize chloroplast or *ubi*ZM1(2) promoter

2338 GTG GCTATTTATC
2351 TATC

Nucleotides 2355-2358 (CGT) connect Region 6 to Region 7a.

Region 7a (SEQ ID NO: 34) Fragment of *pat* gene

2358 GCA GCTGATATGG CCGCGGTTTG TGATATCGTT AACCATTACA
2401 TTGAGACGTC TACAGTGAAC TTTAGGACAG AGCCACAAAC
ACCACAAGAG
2451 TGGATTGATG ATCTAGAGAG GTTGCAAGAT AGATACCCTT
GGTTGGTTGC
2501 TGAGGTTGAG GGTGTTGTGG CTGGTATTGC TTACGCTGGG
CCCTGGAAGG
2551 CTAGGAAC

Region 7b (SEQ ID NO: 35) Fragment of *pat* gene (complement)

2559 CC TCAACCTCAG CAACCAACCA ATGGTATCTA TCTTGCAACC
2601 TCTCTAGATC ATCAATCCAC TCTTGTGGTG TTTGTGGCTC
TGTCCTAAAG
2651 TTCACTGTAG ACGTCTCAAT GTAATGGTTA ACGATATCAC AAACCG

Region 7c (SEQ ID NO: 36) Fragment of *cry*1F gene (complement)

2697 AGAG
2701 AAGAGGGATC T

Region 8 (SEQ ID NO: 37) Fragment of Polylinker

2712 CGAAGCTTC GGCCGGGGCC CATCGATATC CGCGGGCATG
2751 CCTGCAGTGC AGCGTGACCC GGTCGTGCCC CTCTCTAGAG
ATAATGAGCA
2801 TTGCATGTCT AAGTTATAAA AAATTACCA

Region 9 (SEQ ID NO: 25) Full-length insert of PHI8999A

Example 5. Description of the flanking sequence 5' to the insert in maize event TC1507

In order to more fully describe the event TC1507 5' flanking sequence, homology searching was done against the GenBank public databases (release 122, 2/01) using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, pairwise alignments were performed using AlignX (InforMax Inc., Bethesda, MD) to look for homology between the maize event TC1507 flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The TC1507 5' flanking sequence is numbered with base 1 being the furthest 5' to the insert and base 2830 at the starting point of the full-length PHI8999A transgenic insert (see FIG. 1). The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed.

In most cases, similarity searching with the event TC1507 5' flanking sequence resulted in a match to one unique sequence based on a very high percent identity value. Those sequences are identified in Table 1. In addition, there are two regions in the TC1507 5' DNA flanking sequence with high similarity to more than one known sequence. In regions 870-1681 and 2338-2354, the percent identity scores with both sequence fragments are sufficiently high that a single match (homolog) cannot be determined. The two possible homologs for each of these regions are indicated in Table 1.

Highly similar sequences were identified for all but the first 669 base pairs of sequence. Generally, the results of similarity searching indicate high homology with maize genomic sequences 5' to base 1681. The region from base 1682 to the start of the PHI8999A insert at position 2830 contains some fragments associated with the transformation event.

Table 1. Sequence summary for event TC1507 insert

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
1	1-669	669	N/A[1]	N/A	N/A	No significant homology detected
2	670-869	200	90.5	AF123535	52432-52632 (complement)	Undescribed maize genomic sequence
Region	**Location in SEQ ID NO:24**	**Size bp**	**% Identity**	**Homolog**	**Location in homologous sequence**	**Description**
3	870-1681	812	89.4	AF050439	1-801	Fragment of maize Huck-1 retrotransposon 5' LTR[2]
			86.6	AF050438	1-797	Fragment of maize Huck-1 retro-transposon 3' LTR
4	1682-2016	335	100.0	PHI8999A	3149-3483	Fragment of *cry*1F gene
5	2017-2337	321	100.0	X86563	29429-29749	Fragment of maize chloroplast *rpoC2* gene (RNA polymerase beta-2 subunit)
6	2338-2354	17	100.0	X86563	97643-97659	Fragment of maize chloroplast *trnI* gene (tRNA-Ile)
			82.4	PHI8999A	182-197	Fragment of maize *ubi*ZM1(2) promoter
7a	2358-2558	201	100.0	PHI8999A	5320-5475	Fragment of *pat* gene
7b	2559-2696	138	99	PHI8999A	5336-5518 (complement)	Fragment of *pat* gene
7c	2697-2711	15	100.0	PHI8999A	2544-2558 (complement)	Fragment of *cry*1F gene
8	2712-2829	118	100.0	PHI8999A	36-153	Fragment of polylinker region (bases 36-80) and *ubi*ZM1(2) promoter (bases 81-153)
9	2830-	6186	100.0	PHI8999A	11 – 6196	Full-length insert

	9015					of PHI8999A
10	9016-9565	550	100.0	PHI8999A	3906-4456 (complement)	Inverted ORF25 terminator
11	9566-9693	128	100.0	NC_001666	121851-121978 (complement) & 100759-100886	Fragment of maize chloroplast *rps12* rRNA (23S ribosomal RNA)

Region	**Location in SEQ ID NO:24**	**Size bp**	**% Identity**	**Homolog**	**Location in homologous sequence**	**Description**
12	9696-10087	392	99	NC_001666	17091-17483 (complement)	Fragment of maize chloroplast genome
13	10088-10275	188	99	PHI8999A	5333-5520 (complement)	Fragment of *pat* gene
14	10278-10358	81	100	NC_001666	137122-137202 (complement)	Fragment of maize chloroplast "ORF241" – hypothetical protein gene
15	10359-10612	254	N/A[1]	N/A	N/A	No significant homology detected
16	10613-11361	749	N/A[1]	N/A	N/A	No description available

[1] N/A; not applicable
[2] LTR; long terminal repeat

Example 6: Confirmation of the presence of Regions 1, 2, and 3 in an unmodified control corn line

PCR analysis was used to determine if Regions 1, 2, and 3 (Table 1) in the 5' flanking region of Event TC1507 are present in an unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Nine different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified control corn line Hi-II (see Armstrong (1994) *The Maize Handbook*, ed. Freeling and Walbot, Springer-Verlag, New York, pp. 663-671, for information on Hi-II) as outlined in Table 2 using the primer sequences shown in Table 3. Two reactions were designed to amplify DNA within Region 1 of the 5' flanking region from bp 25 to 324 (Reaction A - 300 bp amplicon); and from bp 25 to 480 (Reaction B - 456 bp amplicon). The expected amplicons were present in both the Hi-II unmodified corn

line and in maize event TC1507. One PCR primer pair, Reaction C, spanned Region 2 to Region 3 of the 5' flanking region from bp 759 to 1182 (424 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reaction D, spanned Region 1 to Region 3 of the 5' flanking region from bp 415 to 1182 (768 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reactions E and F were designed as specific primer pairs for the *pat* gene region of the full-length insert of PHI8999A in TC1507 and thus an amplicon in the unmodified Hi-II corn line is not expected. The results indicate that both Reactions E and F are specific for a maize line transformed with a *pat* gene region and produce the expected amplicon, whereas no amplicon was produced in the unmodified Hi-II corn line. Reaction G was also designed as a primer pair that would produce an amplicon of 366 bp in the maize event TC1507 and no amplicon in the unmodified Hi-II corn line.

Reactions H and I were designed as specific primer pairs for TC1507 that would span the end of the transgenic insert into the 5' flanking region. In both Reactions H and I, the reverse primer was located in the ubiquitin promoter region of the full-length PHI8999A insert (Region 9 in Table 1) and the forward primer was located in Region 5, the *rpoC2* gene fragment (see Table 1). Reaction H and Reaction I both produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. These results indicate that both Reactions H and I are specific for the TC1507 event.

The PCR results show that the undescribed sequence (Region 1) is present in the unmodified corn line Hi-II and that Regions 1, 2 and 3, are contiguous in the unmodified corn line Hi-II. The DNA sequences amplified in Reactions A, B, C, and D are not unique to the 5' flanking region of maize event TC1507 but are also present in the unmodified corn line Hi-II.

Table 2. PCR reactions for sequence 5' to the PHI8999A insert in maize event TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
A	25 – 324 bp in TC1507 flanking	300	Region 1	Yes	Yes

	sequence				
B	25 – 480 bp in TC1507 flanking sequence	456	Region 1	Yes	Yes

Reaction	**PCR Amplicon Location**	**Amplicon Size (bp)**	**Region in TC1507 flanking sequence or PHI8999A insert**	**Amplicon present In Hi-II**	**Amplicon present in maize line TC1507**
	bp in TC1507 flanking sequence		3		
D	415 – 1182 bp in TC1507 5' flanking sequence	768	Region 1 to Region3	Yes	Yes
E Not Unique to TC1507	4750 – 5794 bp in PHI8999A	1045	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes
F Not Unique to TC1507	4827 - 5308 bp in PHI8999A	482	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present in Hi-II	Amplicon present in maize line TC1507
Detects cry1F fragment in 5' flanking region	*cry*1F sequence in 5' flanking sequence and in full-length insert of PHI8999A	366	Spans 335 bp *cry*1F sequence in 5' flanking sequence and same sequence in the full-length insert	No	Yes
H Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3069 bp in Region 9 (full-length insert of PHI8999A)	912	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes
I Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3001 bp in Region 9 (full-length insert of PHI8999A)	844	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes

Table 3. PCR primers for sequence 5' to the PHI8999A insert in TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
A	300	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:11	TTGATTGGCAGGTCCGTGGGTC
B	456	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:12	CACAACGGCACAGAAACACGAA
C	424	SEQ ID NO:13	GCGCACCCACCGGAACAAAATG
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
D	768	SEQ ID NO:15	CCTGGCACGCATTGACGCATGT
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
E	1045	SEQ ID NO:6	TAGAGGACCTAACAGAACTCGCCGT
		SEQ ID NO:7	GAGCTGGCAACTCAAAATCCCTTT
Reaction	**Amplicon Size (bp)**	**Primer Pair**	**Primer Sequences 5' to 3'**
F	482	SEQ ID NO:8	AAAATCTTCGTCAACATGGTGGAGC

		SEQ ID NO:9	TAATCTCAACTGGTCTCCTCTCCGG
G	366	SEQ ID NO:19	GGCTCGGACTCGACCTTTCTAT
		SEQ ID NO:20	GCAGTTCTTGAAGAATGAGTGA
H	912	SEQ ID NO:1	GTAGTACTATAGATTATATTATTCGT AGAG
		SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
I	844	SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
		SEQ ID NO:23	CTTCAAACAAGTGTGACAAA

Example 7. Flanking sequence 3' to inserted transgenic DNA in maize event TC1507

Two separate PCR approaches were used to extend the length of the sequence information 3' to the full-length PHI8999A insert in maize event TC1507. In the first approach PCR primer pairs were designed to amplify a product that spanned the junction between the full-length insert and the inverted ORF25 terminator, see FIG. 1 for a depiction of the inverted ORF25 terminator. A forward primer was located at the end of the full-length PHI8999A insert and a series of reverse primers were located at 100 bp intervals in the inverted sequence. In this manner the length of the inverted fragment present in the maize event TC1507 could be determined within a 100 bp region based on the successful PCR reactions. This method indicated the inverted fragment contained the majority of the ORF25 terminator but no Cry1F sequence. PCR fragments were isolated and sequenced from this region.

In the second approach PCR primers were designed to walk out into the flanking DNA sequence from the inverted ORF25 terminator region as determined in the PCR experiment described above. Genomic DNA isolated from two to three individual plants of event TC1507 and an unmodified control corn line was digested with various restriction enzymes and then ligated to adaptors specific for the restriction enzyme used for digestion (Universal Genome Walker™ Kit, Clontech Laboratories, Inc. and Devon *et al.* (1995) *Nucleic Acids Res.* 23:1644-1645). Primary PCR was carried out using an ORF25 terminator specific primer and a primer homologous to the adaptor sequence ligated onto the digested DNA. In order to increase the specificity of the reaction a nested secondary PCR was performed again with another ORF25 terminator specific primer and a secondary primer homologous to the adaptor sequence with the secondary primers being internal to the respective primers used in the primary PCR. Products produced by the nested PCR were analyzed by agarose gel electrophoresis and fragments unique to TC1507 DNA

samples were isolated and sequenced. Fragments were amplified from both the ORF25 terminator contained within the full-length insert and from the targeted (inverted) ORF25 terminator on the 3' end of the full-length PHI8999A insert. Fragments from the full-length insert were of a predicted size based on the knowledge of the restriction enzyme sites located in the full-length insert. Fragments produced from the 3' inverted ORF25 terminator appeared as fragments of unexpected size. Sequence analysis of amplified fragments from the 3' inverted ORF25 terminator resulted in flanking DNA sequence of 1043 bp. Resultant sequence from the above series of genome walking experiments was used to design additional primers to walk further out from the insert into the bordering maize genome with a final 3' flanking sequence, of 2346 bp.

In order to describe the TC1507 3' flanking sequence, homology searching was done against the GenBank public databases using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, alignments were performed using SeqMan 4.05™, Martinez and Needleman-Wunsch alignment algorithms (DNASTAR Inc.) to look for homology between the TC1507 3' flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed. The results of similarity searching for the 3' flanking sequence indicate high homology with three regions of maize chloroplast DNA, a 188 bp fragment of the *pat* gene, and 254 bp of DNA (Region 15, Table 1) with no significant homology. An additional 749 bp (Region 16) beyond Region 15 (see Table 1) was also sequenced. No similarity searching results are available for Region 16.

PCR analysis on control and TC1507 genomic DNA determined that the 254 bp sequence (Region 15, fragment of maize chloroplast "ORF241") is present in the maize genome. The DNA sequence of Region 15 in the 3' flanking region is not unique to the 3' flanking region of maize event TC1507 but is also present in the unmodified control corn line. The TC1507 3' flanking sequence is presented in Example 8 and diagrammed in FIG. 1.

Example 8. Sequence of the region 3' to the end of the full-length insert DNA in maize event TC1507. A description of each region is in Table 1.

Region 10 (SEQ ID NO: 38) Fragment of ORF25 Terminator (complement)

9016 CTCAC TCCGCTTGAT CTTGGCAAAG ATATTTGACG
9051 CATTTATTAG TATGTGTTAA TTTTCATTTG CAGTGCAGTA
TTTTCTATTC
9101 GATCTTTATG TAATTCGTTA CAATTAATAA ATATTCAAAT
CAGATTATTG
9151 ACTGTCATTT GTATCAAATC GTGTTTAATG GATATTTTTA
TTATAATATT
9201 GATGATATCT CAATCAAAAC GTAGATAATA ATAATATTTA
TTTAATATTT
9251 TTGCGTCGCA CAGTGAAAAT CTATATGAGA TTACAAAATA
CCGACAACAT
9301 TATTTAAGAA ACATAGACAT TAACCCTGAG ACTGTTGGAC
ATCAACGGGT
9351 AGATTCCTTC ATGCATAGCA CCTCATTCTT GGGGACAAAA
GCACGGTTTG
9401 GCCGTTCCAT TGCTGCACGA ACGAGCTTTG CTATATCCTC
GGGTTGGATC
9451 ATCTCATCAG GTCCAATCAA ATTTGTCCAA GAACTCATGT
TAGTCGCAAC
9501 GAAACCGGGG CATATGTCGG GTATCTCGAG CTCGCGAAAG
CTTGGCTGCA
9551 GGTCGACGGA TCCTT

Region 11 (SEQ ID NO: 39) Fragment of maize chloroplast *rps12* rRNA gene (complement)

9566 CAACA AAAGGGTACC TGTACCCGAA ACCGACACAG
9601 GTGGGTAGGT AGAGAATACC TAGGGGCGCG AGACAACTCT
CTCTAAGGAA
9651 CTCGGCAAAA TAGCCCCGTA ACTTCGGGAG AAGGGGTGCC CCC

Nucleotides 9694-9695 (CG) connect Region 11 to Region 12.

Region 12 (SEQ ID NO: 40) Fragment of maize chloroplast genome

9696 CTAAC
9701 AATAAACGAA TACGGTTTAT GTATGGATTC CGGTAAAATA
CCGGTACTCG
9751 ATTTCATAAG AGTCGAATAG GAAGTTAAGA TGAGGGTGGT
ATCATCATAA
9801 AAATGGAGTA GTATCCTAAA TTATACTAAT CCACGTATGA
TATGTATGCC
9851 TTTCCTTATC AACCGGAAGT AGTGCAAAAA AAATTCTATA
CTGCACTGCT
9901 CTCTTTTTAC TGAGAAATGC AAAAAAATAA AAGTGAAGTA
AGGGTGCCCC

9951 ATAGATATTT GATCTTGCCT CCTGTCCCCC CCCCCCTTTT
TTCATCAAAA
10001 ATTTCCATGA AAAAAGAAAA GATGAATTTG TCCATTCATT
GAACCCTAGT
10051 TCGGGACTGA CGGGGCTCGA ACCCGCAGCT TCCGCCT

Region 13 (SEQ ID NO: 41) **Fragment of *pat* gene (complement)**

10088 GTT CCTAGCCTTC
10101 CAGGGCCCAG CGTAAGCAAT ACCAGCCACA GCACCCTCAA
CCTCAGCAAC
10151 CAACCAAGGG TATCTATCTT GCAACCTCTC TAGATCATCA
ATCCACTCTT
10201 GTGGTGTTTG TGGCTCTGTC CTAAAGTTCA CTGTAGACGT
CTCAATGTAA
10251 TGGTTAACGA TATCACAAAC CGCGG

Nucleotides 10276-10277 (AA) connect Region 13 to Region 14.

Region 14 (SEQ ID NO: 42) Fragment of maize chloroplast ORF241 (complement)

10278 CAC AAGAACGAAA GCACCTTTTC
10301 ATTCTTTCAT ATACTAGGGG TTTTTACTTG GAAAAGACAA
TGTTCCATAC
10351 TAAAGGAT

Region 15 (SEQ ID NO: 43) **Maize genomic (no significant homology)**

10359 AG CTGCAGAAGC CGCCACCGTC TTGAGGACCT TCCGGGGAGC
10401 CAGACCGGTC GAACCGTGCC TCCACTTGCT AAGGAGAAAG
GGAAAATCAG
10451 GGCCAGGACA TACGAAGGAG GAGCCAGAAC GAAGATATCC
TAAGATACTT
10501 ACTCGCTCCG GGCCATGATC AATCATGCCT GTGGGGAGGT
CTCTCGCACC
10551 TCGATCCATG AAGGTACCAC CGAGGTCTGC CCCGCCGCCG
GCTTCGGTAC
10601 CGTCCTCGCC TT

Region 16 (SEQ ID NO: 44) Maize genomic

10613 GGGCGCCC GAGGCACCCG GGGGATGGAC TGCCCAGGCG
10651 CAGCCACGAC GACCCAAGGA TCACCCTCCT GCGCAGTCGG
CACGAGCAAT
10701 AGTTCTCGGG GAACAGGCAG CTTGGCCTGA CTCCCCGGGG
TCACCTCAAC
10751 TACCTCGGCC GAGGGGTCAA GTACCCCCTC AGTCCGCCCC
CGCTCTTCGG
10801 ACCGGGACCC CGACGTCCCG GCCCCGGATA CCGACGGCAC
CAGCCCGCTC

10851 GGGGGCTGGC TTGACGACCC CTGGCCCAGC CTCAGATCTG
GGCTGAGGCC
10901 GAGGCAGGCG GCCATGTCGT CGTCTTCATC ATCGTCTTCA
TCATCGTCGT
10951 CGTCATCAGG CGTCTCCGGC GACGGCTCCC TTGGGAGCCC
CTCCCTCTCC
11001 TGCCGACGAC GAAGCCTTTC CAAGGCATCC CGAGCCCACG
TCCGCTCGTG
11051 GGCCCGAGCC TTCTTTGCGT CCTTCTTCTC CTTCCTCTTC
TCCGCGGTGA
11101 CCCTCCGCGC AGCTCGGTCC ACCGCATCCT CCGGGACTGG
TGGCAGGGAA
11151 GGCTTGTGAT GCCCTACCTC CTGGAGACAG ACGAAAAGTC
TCAGCTATGA
11201 GAACCGAGGG CAATCTGACG CAAGAAGGAA GAAGGAGCGG
ATACTCACCA
11251 GAGACACGCA CCCGCGATCG GGACGCATTA AGGGCTGGGA
AAAAGTGCCG
11301 GCCTCTAATT TCGCTACCGT GCCGTCCACC CACCTGTGGA
GGTCATCGAT
11351 GGGAAGGGGA A

Example 9. Confirmation of the presence of Region 15 in the unmodified control corn line

PCR analysis was used to determine if the undescribed region of sequence on the end of the 3' flanking sequence (Region 15 in Table 1) is present in the unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Successful PCR amplification of Region 15 in both maize line TC1507 and the unmodified Hi-II control corn line revealed that Region 15 was indeed present in corn genomic DNA. Five different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified Hi-II control corn line as outlined in Table 7 below using the primer sequences shown in Table 8. Three reactions were designed to amplify DNA within Region 15 of the 3' flanking region; Reaction L – producing a 175 bp amplicon, Reaction M – producing a 134 bp amplicon, and Reaction N – producing a 107 bp amplicon. The expected amplicons were present in both the unmodified control corn line and in maize line TC1507. Reactions J and K were designed as specific primer pairs for TC1507 that would span the end of the insert into the 3' flanking region. In Reaction J, the forward primer was located in the *pat* gene fragment on the 3' end of the full-length PHI8999A insert (Region 13 in Table 1) and the reverse primer was located in the undefined Region 15. In Reaction K the forward primer was

located in the chloroplast hypothetical protein gene on the 3' end of the full-length insert (Region 14 in Table 1) and the reverse primer was located in the undefined Region 15. Both Reaction J and Reaction K produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. The results indicate that both Reactions J and K are specific for the TC1507 event.

The PCR results indicate that the undescribed sequence (Region 15) of the 3' flanking sequence of TC1507 is also present in genomic DNA isolated from the unmodified Hi-II control corn line. The DNA sequences amplified in Reactions L, M, and N are not unique to the 3' flanking region of TC1507 but are also present in the unmodified control corn line.

Table 7. PCR reactions for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Region in TC1507 3' flanking sequence	Amplicon present in Control	Amplicon present in maize line TC1507
J	342	Region 13 (*pat* gene fragment) to Region 15	No	Yes
K	252	Region 14 (chloroplast gene) to Region 15	No	Yes
L	175	Region 15	Yes	Yes
M	134	Region 15	Yes	Yes
N	107	Region 15	Yes	Yes

Table 8. PCR primers for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
J	342	SEQ ID NO:3	TGTGGTGTTTGTGGCTCTGTCCTAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
K	252	SEQ ID NO:4	AGCACCTTTTCATTCTTTCATATAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC

L	175	SEQ ID NO:16	AAGCCGCCACCGTCTTGAGGAC CTT
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
M	134	SEQ ID NO:17	GTCGAACCGTGCCTCCACTTGC TAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
N	107	SEQ ID NO:18	AGAAAGGGAAAATCAGGGCCA GGAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC

Example 10. PCR Primers

DNA event specific primer pairs were used to produce an amplicon diagnostic for TC1507. These event primer pairs include, but are not limited to, SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 2 and SEQ ID NO: 23; SEQ ID NO: 3 and SEQ ID NO: 5; and SEQ ID NO: 4 and SEQ ID NO: 5. In addition to these primer pairs, any primer pair derived from SEQ ID NO: 26 and SEQ ID NO: 27 that when used in a DNA amplification reaction produces a DNA amplicon diagnostic for TC1507 is an aspect of the present invention. The amplification conditions for this analysis are illustrated in Table 9, however, any modification of these methods that use DNA primers or complements thereof to produce an amplicon DNA molecule diagnostic for TC1507 is within the ordinary skill of the art. The preferred amplification conditions for reactions utilizing the PCR primers identified in SEQ ID NOS: 1, 2, and 23 are illustrated in Table 10. In addition, control primer pairs, which include SEQ ID NOS: 10 and 11; SEQ ID NOS: 10 and 12; SEQ ID NOS: 13 and 14; SEQ ID NOS: 14 and 15; SEQ ID NOS: 5 and 16; SEQ ID NOS: 5 and 17; and SEQ ID NOS: 5 and 18; for amplification of an endogenous corn gene are included as internal standards for the reaction conditions. Also included are primer pairs that will produce an amplicon in transgenic events containing a *pat* gene (SEQ ID NOS: 6 and 7; SEQ ID NOS: 8 and 9), and a primer pair that will produce an amplicon in transgenic events containing a *cry*1F gene (SEQ ID NOS: 19 and 20).

The analysis of plant tissue DNA extracts to test for the presence of the TC1507 event should include a positive tissue DNA extract control (a DNA sample known to contain the transgenic sequences). A successful amplification of the positive control demonstrates that the PCR was run under conditions which allow for the amplification of

target sequences. A negative, or wild-type, DNA extract control in which the template DNA provided is either genomic DNA prepared from a non-transgenic plant, or is a non-TC1507 transgenic plant, should also be included. Additionally a negative control that contains no template corn DNA extract will be a useful gauge of the reagents and conditions used in the PCR protocol.

Additional DNA primer molecules of sufficient length can be selected from SEQ ID NO: 26 and SEQ ID NO: 27 by those skilled in the art of DNA amplification methods, and conditions optimized for the production of an amplicon that may differ from the methods shown in Table 9 or Table 10 but result in an amplicon diagnostic for event TC1507. The use of these DNA primer sequences with modifications to the methods shown in Table 9 and Table 10 are within the scope of the invention. The amplicon wherein at least one DNA primer molecule of sufficient length derived from SEQ ID NO: 26 and SEQ ID NO: 27 that is diagnostic for event TC1507 is an aspect of the invention. The amplicon wherein at least one DNA primer of sufficient length derived from any of the genetic elements of PHI8999A that is diagnostic for event TC1507 is an aspect of the invention. The assay for the TC1507 amplicon can be performed by using a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler, or by methods and apparatus known to those skilled in the art.

Table 9. PCR Conditions:

Conditions:

Kit used:	Perkin-Elmer AmpliTAQ Gold kit
Volume	Component
5 µl	template (10 ng/µl)
4 µl	2 µl each primer (10 µM)
2 µl	10X PCR Gold Buffer
2 µl	25 mM $MgCl_2$
2 µl	50X dNTP's (10 mM)
0.1 µl	Amplitaq Gold Polymerase
4.9 µl	H_2O
20 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

9 min 92°C
30 cycles:
94°C 30 sec
60°C 30 sec
72°C 1 min
7 min 72°C
Hold 4°C

Table 10. PCR Conditions used with the Advantage®-GC 2 Polymerase Mix:

Conditions:

Kit used:	Advantage®-GC 2 Polymerase Mix
Volume	Component
5 µl	template (10 ng/µl)
5 µl	2.5 µl each primer (10 µM)
10 µl	5x GC2 Buffer
10 µl	GC melt (1.0 M final conc.)
1.5 µl	50X dNTP's (10 mM)
1.0 µl	Advantage GC2 Polymerase
17.5 µl	H_2O
50 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

5 min 94°C
35 cycles:
94°C 1 min
60°C 2 min
72°C 3 min
7 min 72°C
Hold 4°C

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all modifications that are within the spirit and scope of the appended claims.

All publications and published patent documents cited in this specification are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

5

Further Embodiments of the Invention are as follows:

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21.
2. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:22.
3. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:24.
4. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:26.
5. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:27.
6. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising at least a first primer, which recognizes a sequence within SEQ ID NO:21 or within SEQ ID NO:22.
7. The kit of item 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
8. The kit of item 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
9. The kit of item 6, wherein the first primer recognizes a sequence within SEQ ID NO:21.
10. The kit of item 6, wherein the first primer recognizes a sequence within SEQ ID NO:22.
11. The kit of item 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.
12. The kit of item 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.
13. The kit of item 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.
14. The kit of item 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

15. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 26.

16. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 27.

17. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with SEQ ID NO:21 and SEQ ID NO:25, contiguous therewith.

18. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

19. A DNA detection kit comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny.

20. A DNA construct comprising: a first and second expression cassette, wherein said first expression cassette in operable linkage comprises

(a) a maize ubiquitin promoter;

(b) a 5' untranslated exon of a maize ubiquitin gene;

(c) a maize ubiquitin intron;

(d) a Cry1F encoding DNA molecule; and

(e) a 3' ORF25 transcriptional terminator; and

said second expression cassette comprising in operable linkage

(i) a CaMV 35S promoter;

(ii) a *pat* encoding DNA molecule; and

(iii) a 3' transcriptional terminator from (CaMV) 35S.

21. A plant comprising the DNA construct of item 20.

22. A plant of item 21, wherein said plant is a corn plant.

23. A method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22.

24. The method of item 23, further comprising amplifying a DNA fragment from a nucleic acid present in said biological sample using a polymerase chain reaction with at least two primers, wherein said first primer recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, and a second primer recognizes a sequence within SEQ ID NO:22 or SEQ ID NO: 25.

25. The method of item 24, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

26. The method of item 23, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

27. The method of item 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively.

28. The method of item 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

29. The method of item 26, wherein said first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively.

30. The method of item 26, wherein said first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively.

31. The method of item 27, comprising amplifying a fragment of about 912 bp using a TC1507 PCR identification protocol.

32. The method of item 28, comprising amplifying a fragment of about 844 bp using a TC1507 PCR identification protocol.

33. The method of item 29, comprising amplifying a fragment of about 342 bp using a TC1507 PCR identification protocol.

34. The method of item 30, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol.

35. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:1 and SEQ ID NO:2 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

36. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:2 and SEQ ID NO:23 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

37. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:3 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

38. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:4 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

39. An isolated DNA molecule comprising the amplicon produced by the method of item 35.

40. An isolated DNA molecule comprising the amplicon produced by the method of item 36.

41. An isolated DNA molecule comprising the amplicon produced by the method of item 37.

42. An isolated DNA molecule comprising the amplicon produced by the method of item 38.

43. A method of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:

a. contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;

b. subjecting the sample and probe to stringent hybridization conditions; and

c. detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event.

44. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 or its complement.

45. An isolated DNA nucleotide primer sequence of item 44 comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement.

46. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:26 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.

47. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.

48. An isolated DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

49. A method for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample.

50. A method for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot.

51. An insect resistant corn plant, or parts thereof, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

52. A descent plant of the insect resistant corn plant of item 51 wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

53. Seed of a plant of items 51 or 52.

54. A method of producing an insect resistant corn plant comprising breeding with a plant of items 51 or 52, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

55. An isolated DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

56. A pair of isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce an amplicon diagnostic for event TC1507.

57. The pair of isolated DNA sequences of item 56 wherein each is chosen from SEQ ID NO:26.

58. The pair of isolated DNA sequences of item 56 wherein each is chosen from SEQ ID NO:27.

59. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is on opposite sides of a sequence diagnostic for said TC1507 event insertion;

(b) contacting a sample of said corn tissue with said primer pair;

(c) performing DNA amplification and analyzing for an amplicons.

60. The method of item 59 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

61. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) subjecting said sample and probe to stringent hybridization conditions; and

(c) analyzing for hybridization of the probe.

62. A DNA detection kit comprising a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

63. A DNA detection kit comprising a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion.

64. The DNA detection kit of item 61 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

65. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

66. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

CLAIMS

1. An insect resistant corn plant or parts thereof, or corn seed, comprising event TC1507.

2. An insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

3. The plant or seed of claims 1 or 2, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising event TC1507 or SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

4. An insect resistant corn plant or parts thereof, or a descent plant of said corn plant, or corn seed, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the genome of the plant or seed.

5. A method of producing an insect resistant corn plant comprising breeding with a plant of claim 4, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

6. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

7. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising at least a first primer, which recognizes a sequence within SEQ ID NO:21 or within SEQ ID NO:22; preferably

(a) further comprising at least a second primer which recognizes:

(i) a sequence within SEQ ID NO:25; more preferably wherein said at least first and second primers comprises the sequence of SEQ ID NO:1 and SEQ ID NO:2 respectively, or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively; or

(ii) a sequence within SEQ ID: 22; more preferably wherein said at least first and second primers comprises the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively, or the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively; or

(b) wherein the first primer recognizes a sequence within SEQ ID NO:21 or 22.

8. A DNA detection kit:

A. specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 26 or 27; or

B. comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny; or

C. comprising:

(a) a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the

group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion, optionally wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

9. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with:

(a) SEQ ID NO:21 and SEQ ID NO:25, contiguous therewith; or

(b) SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

10. A method:

A. for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22; preferably

(a) further comprising amplifying a DNA fragment from a nucleic acid present in said biological sample using a polymerase chain reaction with at least two primers, wherein said first primer recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, and a second primer recognizes a sequence within SEQ ID NO:22 or SEQ ID NO: 25; more preferably wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25; wherein said first and second primers comprise:

(i) the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively, comprising amplifying a fragment of about 912 bp using TC1507 PCR identification protocol; or

(ii) the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively, comprising amplifying a fragment of about 844 bp using TC1507 PCR identification protocol; or

(b) wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22; more preferably wherein said first and second primers comprise:

(i) the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively, comprising amplifying a fragment of about 342 bp using TC1507 PCR identification protocol; or

(ii) the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol; or

B. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:1 and SEQ ID NO:2 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

C. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:2 and SEQ ID NO:23 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

D. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:3 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

E. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:4 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

F. of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:

(a) contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;

(b) subjecting the sample and probe to stringent hybridization conditions; and

(c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event; or

G. for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample; or

H. for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot; or

I. of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is on opposite sides of a sequence diagnostic for said TC1507 event insertion;

(b) contacting a sample of said corn tissue with said primer pair;

(c) performing DNA amplification and analyzing for an amplicons, optionally wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; or

J. of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) subjecting said sample and probe to stringent hybridization conditions; and

(c) analyzing for hybridization of the probe.

11. An isolated DNA molecule comprising:

(a) the amplicon produced by the method of claim 10B;

(b) the amplicon produced by the method of claim 10C;

(c) the amplicon produced by the method of claim 10D; or

(d) the amplicon produced by the method of claim 10E.

12. An isolated:

A. DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 or its complement; optionally further comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement;

B. DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; or

C. DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

13. A pair of:

A. DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:26 or 27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof; or

B. isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce

an amplicon diagnostic for event TC1507; optionally wherein each is chosen from SEQ ID NO:26 or 27.

14. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

15. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

CLAIMS

1. An insect resistant corn plant or parts thereof, or corn seed, comprising event TC1507.

2. An insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

3. The plant or seed of claims 1 or 2, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising event TC1507 or SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

4~~5~~1. An insect resistant corn plant~~,~~ or parts thereof, or a descent plant of said corn plant, or corn seed, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the ~~plant's~~ genome of the plant or seed.

5~~5~~4. A method of producing an insect resistant corn plant comprising breeding with a plant of claim 4~~51 or 52~~, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

6~~1~~. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21~~,~~, 22, 24, 26 or 27.

~~2. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:22.~~

~~3. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:24.~~

~~4. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:26.~~

~~5. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:27.~~

76. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising at least a first primer, which recognizes a sequence within SEQ ID NO:21 or within SEQ ID NO:22.~~.~~; preferably

~~7. The kit of claim 6,~~ (a) further comprising at least a second primer which recognizes:

(i) a sequence within SEQ ID NO:25.~~.~~; more preferably wherein said at least first and second primers comprises the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively, or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively; or

(ii) a sequence with SEQ ID NO: 22; more preferably wherein said at least first and second primers comprises the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively, or the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively; or

~~8. The kit of claim 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.~~

~~9. The kit of claim 6,~~ (b) wherein the first primer recognizes a sequence within SEQ ID NO:21~~.~~ or 22.

~~10. The kit of claim 6, wherein the first primer recognizes a sequence within SEQ ID NO:22.~~

~~11. The kit of claim 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.~~

~~12. The kit of claim 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.~~

~~13. The kit of claim 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.~~

~~14. The kit of claim 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.~~

8~~15~~. A DNA detection kit:

A. specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 26~~.~~ or 27; or

B. comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny; or

C. comprising:

(a) a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion, optionally wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

~~16. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 27.~~

9~~17~~. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with:

(a) ~~-~~SEQ ID NO:21 and SEQ ID NO:25, contiguous therewith~~.~~; or

~~18. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with~~ (b) SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

~~19. A DNA detection kit comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny.~~

~~20. A DNA construct comprising: a first and second expression cassette, wherein said first expression cassette in operable linkage comprises~~

~~(a) a maize ubiquitin promoter;~~

~~(b) a 5' untranslated exon of a maize ubiquitin gene;~~

~~(c) a maize ubiquitin intron;~~

~~(d) a Cry1F encoding DNA molecule; and~~

~~(e) a 3' ORF25 transcriptional terminator; and~~

~~said second expression cassette comprising in operable linkage~~

~~(i) a CaMV 35S promoter;~~

~~(ii) a *pat* encoding DNA molecule; and~~

~~(iii) a 3' transcriptional terminator from (CaMV) 35S.~~

~~21. A plant comprising the DNA construct of claim 20.~~

~~22. A plant of claim 21, wherein said plant is a corn plant.~~

10~~23~~. A method:

A. ~~-~~for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22~~.~~; preferably

~~24. The method of claim 23,~~ (a) further comprising amplifying a DNA fragment from a nucleic acid present in said biological sample using a polymerase chain reaction with at least two primers, wherein said first primer recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, and a second primer recognizes a sequence within SEQ ID NO:22 or SEQ ID NO: 25~~.~~;

~~25. The method of claim 24,~~ more preferably wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25~~.~~;

wherein said first and second primers comprise:

(i) the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively, comprising amplifying a fragment of about 912 bp using TC1507 PCR identification protocol; or

(ii) the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively, comprising amplifying a fragment of about 844 bp using TC1507 PCR identification protocol; or

~~26. The method of claim 23,~~ (b) wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22~~.~~;

~~27. The method of claim 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively.~~

~~28. The method of claim 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.~~

~~29. The method of claim 26,~~ more preferably wherein said first and second primers comprise:

(i) ~~the~~ the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively~~.~~, comprising amplifying a fragment of about 342 bp using TC1507 PCR identification protocol; or

~~30. The method of claim 26, wherein said first and second primers comprise~~

(ii) the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively~~.~~, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol; or

~~31. The method of claim 27, comprising amplifying a fragment of about 912 bp using a TC1507 PCR identification protocol.~~

~~32. The method of claim 28, comprising amplifying a fragment of about 844 bp using a TC1507 PCR identification protocol.~~

~~33. The method of claim 29, comprising amplifying a fragment of about 342 bp using a TC1507 PCR identification protocol.~~

~~34. The method of claim 30, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol.~~

B. ~~35. A method~~ of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:1 and SEQ ID NO:2 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.; or

C.36. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:2 and SEQ ID NO:23 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.; or

D. 37. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:3 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.; or

E. 38. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:4 and SEQ ID NO:5 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507~~.~~; or

F. of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:

a. contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;

b. subjecting the sample and probe to stringent hybridization conditions; and

c. detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event; or

G. for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample; or

H. for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot; or

I. of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is on opposite sides of a sequence diagnostic for said TC1507 event insertion;

(b) contacting a sample of said corn tissue with said primer pair;

(c) performing DNA amplification and analyzing for an amplicons,

optionally wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; or

J. of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) subjecting said sample and probe to stringent hybridization conditions; and

(c) analyzing for hybridization of the probe.

1139. An isolated DNA molecule comprising:

(a) the amplicon produced by the method of claim 10B35.;

~~40. An isolated DNA molecule comprising~~ (b) the amplicon produced by the method of claim 10C36.;

~~41. An isolated DNA molecule comprising~~ (c) the amplicon produced by the method of claim 10D37.; or

~~42. An isolated DNA molecule comprising~~ (d) the amplicon produced by the method of claim 10E38.

~~43. A method of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:~~

~~a. contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;~~

~~b. subjecting the sample and probe to stringent hybridization conditions; and~~

~~c. detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event.~~

1244. An isolated ~~DNA~~:

A. DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 or its complement;~~.~~ optionally further comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement;

B. DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; or

C. DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

~~45. An isolated DNA nucleotide primer sequence of claim 44 comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement.~~

1346. A pair of:

A, ~~-~~DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:26 or 27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof~~.~~; or

B. isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce an amplicon diagnostic for event TC1507; optionally wherein each is chosen from SEQ ID NO:26 or 27.

~~47. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.~~

48. An isolated DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

49. A method for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample.

50. A method for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot.

52. A descent plant of the insect resistant corn plant of claim 51 wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

53. Seed of a plant of claim 51 or 52.

55. An isolated DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

56. A pair of isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce an amplicon diagnostic for event TC1507.

57. The pair of isolated DNA sequences of claim 56 wherein each is chosen from SEQ ID NO:26.

58. The pair of isolated DNA sequences of claim 56 wherein each is chosen from SEQ ID NO:27.

59. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is

~~on opposite sides of a sequence diagnostic for said TC1507 event insertion;~~

~~(a) contacting a sample of said corn tissue with said primer pair;~~

~~(b) performing DNA amplification and analyzing for an amplicons.~~

~~60. The method of claim 59 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.~~

~~61. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:~~

~~(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;~~

~~(a) subjecting said sample and probe to stringent hybridization conditions; and~~

~~(b) analyzing for hybridization of the probe.~~

~~62. A DNA detection kit comprising a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.~~

~~63. A DNA detection kit comprising a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion.~~

~~64. The DNA detection kit of claim 61 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.~~

1465. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

1566. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of your request for grant of a European patent as follows:

Submission number	3505010
Application number	EP15168051.9
File No. to be used for priority declarations	EP15168051
Date of receipt	18 May 2015
Your reference	N96314A-EP
Applicant	Dow AgroSciences, LLC
Country	US
Title	CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

Documents submitted	
package-data.xml	ep-request.xml
application-body.xml	ep-request.pdf (5 p.)
SPECEPO-1.pdf\N96314A-EP AXB KKT mqr Spec as filed - May15.pdf (61 p.)	SPECEPO-2.pdf\N96314A-EP AXB KKT mqr Figure filed - May15.pdf (1 p.)
SEQLTXT.txt\N96314A-EP AXB KKT Sequence Listing - May15.txt	OTHER-1.pdf\N96314A-EP AXB KKT mqr Track change claims - May15.pdf (12 p.)
OTHER-2.pdf\N96314-EP AXB KKT mqr Search Report.pdf (3 p.)	OTHER-3.pdf\N96314A-EP AXB KKT mqr File new Div App - May15.pdf (3 p.)
f1002-1.pdf (4 p.)	

Submitted by	CN=Andrew Bentham 23464

Method of submission	Online
Date and time receipt generated	18 May 2015, 20:04 (CEST)
Message Digest	F0:E6:27:B8:04:42:98:5D:42:4A:4F:48:E7:FF:C3:37:E0:56:C7:A4

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

ABSTRACT OF THE DISCLOSURE

The invention provides DNA compositions that relate to transgenic insect resistant maize plants. Also provided are assays for detecting the presence of the maize TC1507 event based on the DNA sequence of the recombinant construct inserted into the maize genome and the DNA sequences flanking the insertion site. Kits and conditions useful in conducting the assays are provided.

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Hartnett Locke, Mary E.
31 Millwood Drive
Mickleton, NJ 08056
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Sanders, Craig D.
15 Penny Lane
Bear, DE 19701
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Rudert, Marjorie L.
1364 274th Lane
Boone, IA 50036
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Koziel, Michael G.
1601 Liatris Lane
Raleigh, NC 27613
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Shao, Aihua
501 Malinmor Drive
St Charles, MO 63304
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Tagliani, Laura A.
4100 Huntsman Drive
Zionsville, IN 46077
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Meyer, Terry E.
4338 101st Street
Urbandale, IA 50322
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Moellenbeck, Daniel
11655 N.W. 107th Court
Granger, IA 50109
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Stucker, David S.
9028 Timberwood Drive
Johnston, IA 50131
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Stelman, Steven J.
11221 Via Madonna
Santee, CA 92071
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Gupta, Manju
13463 Winamac Court
Carmel, IN 46032
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Van Zante, William M.
4424 73rd Place
Urbandale, IA 50322
ETATS-UNIS D'AMERIQUE

Date
..

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bing, James W.
1565 N.E. 118th Avenue
Ankeny, IA 50021
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Ritchie, Steven W.
16218 Wright Circle
Omaha, NE 68130
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Cressman, Robert F., Jr.
2502 Turnstone Drive
Wilmington, DE 19805
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Barbour, Eric
6075 Annapolis Lanc N.
Plymouth, MN 55446
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Narva, Kenneth E.
2856 Via Conquistador
Carlsbad, CA 92009
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Keaschall, Joseph W.
13563 Summit Drive
Clive, IA 50325
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Hondred, David
1432 N.W. 72nd Avenue
Ankeny, IA 50021
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Nirunsuksiri, Wilas
515 Aaby Drive
Auburn, WA 98001
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Cardineau, Guy A.
18 West Cottage Lane
Tempe, AZ 85282
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Meyer, Terry E.
4338 101st Street
Urbandale, IA 50322
ETATS-UNIS D'AMERIQUE

Date
10.07.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./4338 101st Street/Urbandale, IA 50322/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

PRIORITY
PRIORITAIRE

Port Betaald
Port Payé
Pays-Bas
postnl

EPA EPO OEB
tel: (070)3403060
10 -08- 2015
Mailroom 11

X 100 NEE 1009414I0107/17/15
FORWARD TIME EXP RTN TO SEND
MEYER
2507 REDFREE DR
W LAFAYETTE IN 47906-4892

RETURN TO SENDER

If undeliverable, please return to:
European Patent Office, P.O. Box 5818, 2280 HV Rijswijk, Pays-Bas



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Meyer, Terry E.
2507 Redfree Dr
W Lafayette, IN 47906-4892
ETATS-UNIS D'AMERIQUE

Date
24.08.15

Reference	Application No./Patent No.
	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Designation as inventor - communication under Rule 19(3) EPC

You have been designated as inventor in the above-mentioned European patent application. Below you will find the data contained in the designation of inventor and further data mentioned in Rule 143(1) EPC:

DATE OF FILING : 29.04.04

PRIORITY : US/02.05.03/ USP 467772

TITLE : CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

DESIGNATED STATES : AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LI LU MC NL PL PT RO SE SI SK TR

INVENTOR (PUBLISHED = 1, NOT PUBLISHED = 0):

1/Bing, James W./1565 N.E. 118th Avenue/Ankeny, IA 50021/US
1/Barbour, Eric/6075 Annapolis Lanc N./Plymouth, MN 55446/US
1/Cardineau, Guy A./18 West Cottage Lane/Tempe, AZ 85282/US
1/Cressman, Robert F., Jr./2502 Turnstone Drive/Wilmington, DE 19805/US
1/Gupta, Manju/13463 Winamac Court/Carmel, IN 46032/US
1/Hartnett Locke, Mary E./31 Millwood Drive/Mickleton, NJ 08056/US
1/Hondred, David/1432 N.W. 72nd Avenue/Ankeny, IA 50021/US
1/Keaschall, Joseph W./13563 Summit Drive/Clive, IA 50325/US
1/Koziel, Michael G./1601 Liatris Lane/Raleigh, NC 27613/US
1/Meyer, Terry E./2507 Redfree Dr/W Lafayette, IN 47906-4892/US
1/Moellenbeck, Daniel/11655 N.W. 107th Court/Granger, IA 50109/US
1/Narva, Kenneth E./2856 Via Conquistador/Carlsbad, CA 92009/US
1/Nirunsuksiri, Wilas/515 Aaby Drive/Auburn, WA 98001/US
1/Ritchie, Steven W./16218 Wright Circle/Omaha, NE 68130/US
1/Rudert, Marjorie L./1364 274th Lane/Boone, IA 50036/US
1/Sanders, Craig D./15 Penny Lane/Bear, DE 19701/US
1/Shao, Aihua/501 Malinmor Drive/St Charles, MO 63304/US
1/Stelman, Steven J./11221 Via Madonna/Santee, CA 92071/US
1/Stucker, David S./9028 Timberwood Drive/Johnston, IA 50131/US
1/Tagliani, Laura A./4100 Huntsman Drive/Zionsville, IN 46077/US
1/Van Zante, William M./4424 73rd Place/Urbandale, IA 50322/US

DECLARATION UNDER ARTICLE 81 EPC:
The applicant(s) has (have) acquired the right to the European patent.

Receiving Section



Application No.: 15168051.9

The search has started on 21.09.15 (see Notice from the European Patent Office dated 03/2013, OJ EPO 2013, 153).

Receiving Section

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 15 16 8051

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	ANONYMOUS: "DAS-01507-1 (TC1507)", INTERNET ARTICLE , 17 October 2002 (2002-10-17), XP002312770, Retrieved from the Internet: URL:http://www.agbios.com/dbase.php?action=showprod&data=TC1507&frmat=long [retrieved on 2005-01-04]	8,10	INV. C12Q1/68 C12N15/82
A	* the whole document *	1,9,11, 13-15	

X	Anonymous: "PUBAQ15TD ZM_0.6_1.0_KB Zea mays genomic clone ZMMBTa012C05, genomic s - GSS - NCBI", , 5 February 2003 (2003-02-05), XP055215477, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/nucgss/BZ677673 [retrieved on 2015-09-23] * abstract; sequence 1 * * the whole document *	12	
	-----		TECHNICAL FIELDS SEARCHED (IPC)
X	Anonymous: "ig56e02.b1 WGS-ZmaysF (DH5a methyl filtered) Zea mays genomic clone ig - GSS - NCBI", , 16 January 2003 (2003-01-16), XP055215479, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/nucgss/BZ616018 [retrieved on 2015-09-23] * sequence 1 * * abstract * * the whole document *	12	C12Q

	-/--		

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	24 September 2015	Helliot, Bertrand

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

2

EPO FORM 1503 03.82 (P04C01)

Europäisches Patentamt
European Patent Office
Office européen des brevets

EUROPEAN SEARCH REPORT

Application Number
EP 15 16 8051

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
X	Anonymous: "GSN:AAN80062", , 21 January 1987 (1987-01-21), XP055215482, Retrieved from the Internet: URL:http://ibis.internal.epo.org/exam/dbfetch.jsp?id=GSN%3AAAN80062 [retrieved on 2015-09-23] * sequence 1 * * abstract * * the whole document * -----	12	
X	Anonymous: "EM_STD:D28338", , 27 January 1995 (1995-01-27), XP055215485, Retrieved from the Internet: URL:http://ibis.internal.epo.org/exam/dbfetch.jsp?id=EM_STD%3AD28338 [retrieved on 2015-09-23] * sequence 1 * * abstract * * the whole document * -----	12	
X	Anonymous: "PUBAQ15TD ZM_0.6_1.0_KB Zea mays genomic clone ZMMBTa012C05, genomic s - GSS - NCBI", , 3 February 2003 (2003-02-03), XP055215487, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/nucgss/BZ677673 [retrieved on 2015-09-23] * sequence 1 * * abstract * * the whole document * -----	12	

TECHNICAL FIELDS SEARCHED (IPC)

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
Munich	24 September 2015	Helliot, Bertrand

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

2

EPO FORM 1503 03.82 (P04C01)

The examination is being carried out on the **following application documents**

Description, Pages

1-52 as originally filed

Sequence listings, SEQ ID NO

1-57 as originally filed

Claims, Numbers

1-15 as originally filed

Drawings, Sheets

1/1 as originally filed

1 INTRODUCTION

1.1 The following documents (D1-D6) are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D1 ANONYMOUS: "DAS-O15O7-1 (TC1507)",
INTERNET ARTICLE
, 17 October 2002 (2002-10-17), XP002312770,
Retrieved from the Internet:
URL:http://www.agbios.com/dbase.php?
action=showprod&data=TC1507&frmat=long
[retrieved on 2005-01-04]

D2 Anonymous: "PUBAQ15TD ZM_0.6_1.0_KB Zea mays genomic clone ZMMBTa012C05, genomic s - GSS - NCBI",
, 22 September 2015 (2015-09-22), XP055215477,

Retrieved from the Internet:
URL:http://www.ncbi.nlm.nih.gov/nucgss/BZ677673
[retrieved on 2015-09-23]

D3 Anonymous: "ig56e02.b1 WGS-ZmaysF (DH5a methyl filtered) Zea mays genomic clone ig - GSS - NCBI",
, 22 September 2015 (2015-09-22), XP055215479,
Retrieved from the Internet:
URL:http://www.ncbi.nlm.nih.gov/nucgss/BZ616018
[retrieved on 2015-09-23]

D4 Anonymous: "GSN:AAN80062",
, 22 September 2015 (2015-09-22), XP055215482,
Retrieved from the Internet:
URL:http://ibis.internal.epo.org/exam/dbfetch.jsp?id=GSN%3AAAN80062
[retrieved on 2015-09-23]

D5 Anonymous: "EM_STD:D28338",
, 22 September 2015 (2015-09-22), XP055215485,
Retrieved from the Internet:
URL:http://ibis.internal.epo.org/exam/dbfetch.jsp?id=EM_STD%3AD28338
[retrieved on 2015-09-23]

D6 Anonymous: "PUBAQ15TD ZM_0.6_1.0_KB Zea mays genomic clone ZMMBTa012C05, genomic s - GSS - NCBI",
, 22 September 2015 (2015-09-22), XP055215487,
Retrieved from the Internet:
URL:http://www.ncbi.nlm.nih.gov/nucgss/BZ677673
[retrieved on 2015-09-23]

1.2 The present application relates to the insect resistant corn (*Zea maya*) plant TC1507, also referred to as maize line TC1507 or maize event TC1507, and to the DNA plant expression construct of corn plant TC1507 and the detection of the transgene/flanking insertion region in corn plant TC1507 and progeny thereof.

2 ART. 76(1)

Art. 76(1) EPC states that an European divisional application shall be filed only in respect of subject-matter which does not extend beyond the content of the earlier application.

The Guidelines teach that the parent application must be pending when a divisional application is filed (see G1/09). An application is pending up to (but not including) the date that the European Patent Bulletin mentions the grant of the patent (OJ EPO 2002, 112).

In the present case, the grant of European Patent EP 1 620 571 to Dow AgroSciences, LLC is mentioned in the European Bulletin 2015/27 published on 01.07.2015. The patent is based upon the European Patent Application EP-04751097.9 which correspond to the earlier application of the present application filed on 18.05.2015.

As the earlier application was still pending at the date of filing of the present application, and in view of the sections in the earlier application (EP 04 751 097) which were cited by the Applicant in his letter by 18.05.2015, the divisional application does not offend against the provisions of Art. 76(1) EPC.

3 CLARITY (Art. 84 EPC)

3.1 The subject-matter of independent **claim 1,** which relates to an insect resistant corn plant or parts thereof or corn seed comprising event TC1507 , lacks clarity in the sense of Article 84 EPC because the term TC1507 is an arbitrary internal designation which gives a vague and unclear definition.

3.2 **Claim 10-F** relates to a method of detecting the presence of DNA corresponding to the TC 1507 event in a sample, the method comprising:

(a) contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC 1507 maize plant DNA;

(b) subjecting the sample and probe to stringent hybridization conditions; and

(c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event.

Present **claim 10-F** does not meet the requirements of clarity and support (Article 84 EPC) in that the matter for which protection is sought is not defined. The claim attempts to define the subject-matter only in functional terms since **claim 10-F** is silent as to any structural feature of the claimed polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC 1507 maize plant DNA. In absence of any structural feature relating to the said probe, present **claim 10-F** does not meet the requirements of support (Article 84 EPC) because it merely amounts to an invitation to carry out a research program leaving to the skilled person the entire burden of identifying the relevant probe.

4 NON PATENTABILITY (Art. 53(b) EPC)

Present **claim 5** relates to a method of producing an insect resistant corn plant. The method comprises a breeding step.

The Guidelines G-II, 5.4 teach that the list of exceptions to patentability under Art. 53(b) also includes "plant or animal varieties or essentially biological processes for the production of plants or animals".

Moreover, the Guidelines G-II, 5.4.2 further stress a process for the production of plants or animals which is based on the sexual crossing of whole genomes and on the subsequent selection of plants or animals is excluded from patentability as being essentially biological, even if other technical steps relating to the preparation of the plant or animal or its further treatment are present in the claim before or after the crossing and selection steps (see G 1/08). To take some examples, a method of crossing, inter-breeding, or selectively breeding, say, horses involving merely selecting for breeding and bringing together those animals (or their gametes) having certain characteristics would be essentially biological and therefore unpatentable. This method remains essentially biological and unpatentable even if it contains an additional feature of a technical nature, for example the use of genetic molecular markers to select either parent or progeny.

5 NOVELTY (Art. 52(1) and Art. 54(1)(2) EPC)

5.1 D1 relates to the transgenic maize line TC1507. However, D1 does not discloses the sequence of the regions surrounding the transgenic event.

However, in such a case, the Enlarged Board of Appeal held in the decision G1/92 that the chemical composition of a product forms part of the state of the art when the product as such is available to the public and can be analysed and reproduced by the skilled person, irrespective of whether or not particular reasons can be identified for analysing the composition.

With respect to the parental application (EP-04751097.9), the Applicant provided evidence (see letter dated 16.10.2014) showing that the maize TC1507 was not available to the public for scientific purposes. The skilled person was thus not in the position to analyse and reproduce said transgenic maize. Accordingly, the subject-matter of independent **claim 1** is considered novel (Art. 54(1) and (2) EPC).

5.2 Even if **claims 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14 and 15** are draft as independent claims, they all relate to the transgenic maize line TC1507 of claim 1 and to sequences for detecting it . Accordingly, said claims are also considered novel (Art. 54(1) and (2) EPC).

5.3 The present application does not meet the requirements of Art. 52(1) EPC, because the subject-matter of **claim 12** is not new over D2-D6 (Art. 54(1) and (2) EPC). Each of the documents D2-D6 discloses respectively an isolated DNA sequence comprising a sequence corresponding to the sequence listed as SEQ ID N° 1, 2, 3, 4 and 23.

6 INVENTIVE STEP (Art. 52(1) and 56 EPC)

6.1 D1 relates to the transgenic maize line TC1507.

The problem to be solved by the present application is to provide compositions and methods for identifying the transgenic corn plant TC1507, which methods are based on primers and/or probes which specifically recognised the 5'- and/or 3'-flanking sequence(s) of the TC1507 maize.

The sequences listed as SEQ ID N° 21, 22, 26 or 27 are the 5'- and/or 3'-flanking sequence(s) of the TC1507 maize.

Since the maize TC1507 was not available to the public for scientific purpose (see letter of the Applicant dated 16-10-2014 in the ealier application), it was thus not possible for the skilled person to sequence corresponding to the event TC1507 and to the region surrounding the transgenic event TC1507. Accordingly, an inventive step can be acknowledged for the plants, part thereof, seed, descent plant as disclosed in **claims 1-4**.

6.2 Moreover, an inventive step can be acknowledged for compositions and methods for identifying the transgenic corn plant TC1507, which methods and compositions are based on primers and/or probes which specifically recognised the sequence SEQ ID N° 24 and the sequences 5'- and/or 3'-flanking sequence(s) of the TC1507 maize listed as SEQ ID N° 21, 22, 24, 26 or 27. Accordingly, an inventive step can be acknowledged for the kit as disclosed in **claims 7, 8-A and 14**.

6.3 Moreover, DNA molecules consisting of SEQ ID N0: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof comprise at least one junction sequence of TC1507 selected from the group; wherein a junction sequence spans the junction between heterologous DNA inserted into the genome and the DNA from the corn cell flanking the insertion site, i.e. flanking DNA, and is diagnostic for the TC1507 event.

Accordingly, an inventive step can be acknowledged for the kit as disclosed in **claims 8-C**.

6.4 The same applies to **claim 9**.

6.5 The Guidelines (see G.L., F-IV, 4.9) teach that expressions like "preferably", "for example", "such as" or "more particularly" should be looked at carefully to ensure that they do not introduce ambiguity. Expressions of this kind have no limiting effect on the scope of a claim; that is to say, the feature following any such expression is to be regarded as entirely optional.

Present **claim 10-A** relates thus to a method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22.

In example 6, PCR analysis was used to determine if Regions 1, 2, and 3 (Table 1) in the 5' flanking region of Event TC1507 are present in an unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Two reactions were designed to amplify DNA within Region 1 of the 5' flanking region from bp 25 to 324 (Reaction A - 300 bp amplicon); and from bp 25 to 480 (Reaction B - 456 bp amplicon). The expected amplicons were present in both the Hi-IIunmodified corn line and in maize event TC1507.One PCR primer pair, Reaction C, spanned Region 2 to Region 3 of the 5' flanking region from bp 759 to 1182 (424 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reaction D, spaimed Region 1 to Region 3 of the 5' flanking region from bp 415 to 1182 (768 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507.

In example 9, PCR analysis was used to determine if the undescribed region of sequence on the end of the 3' flanking sequence (Region 15 in Table 1) is present in the unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Successful PCR amplification of Region 15 in both maize line TC1507 and the unmodified Hi-II control corn line revealed that Region 15 was indeed present in corn genomic DNA. Three reactions were designed to amplify DNA within Region 15 of the 3' flanking region; Reaction L - producing a 175 bp amplicon, Reaction M - producing a 134 bp amplicon, and Reaction N - producing a 107 bp amplicon. The expected amplicons were present in both the unmodified control corn line and in maize line TC1507 (see Tab. 7).

Thus, a probe hybridizing to the amplicons resulting from reactions A, B, C, D, L, M and N does not solve the technical problem of identifying event TC1507. Accordingly, the subject-matter of **claims 10-A, 10-G, 10-H** does not involve an inventive step over the entire scope insofar (Art. 56 EPC). The present application does not meet the requirement of Art. 52(1) EPC.

6.6 For this reason, the kit of **claim 8-B** is not inventive (Art. 56 EPC).

6.7 In examples 9 and 10, DNA event specific primer pairs were used to produce an amplicon diagnostic for TC1507. These event primer pairs include, but are not limited to, SEQ ID NO: 1 and SEQ ID NO: 2 (reaction H); SEQ ID NO: 2 and SEQ ID NO: 23 (reaction I); SEQ ID NO: 3 and SEQ ID NO: 5 (reaction J); and SEQ ID NO: 4 and SEQ ID NO: 5 (reaction K).

Reactions H, I, J and K produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. These results indicate that Reactions H, I, J and K are specific for the TC1507 event.

Accordingly, an inventive step can be acknowledged for the method as disclosed in **claims 10-B, 10-C, 10-D and 10-E**.

6.8 In view of the clarity problem as raised unter item 3.2 herein above, it is considered that present **claim 10-F** does not solve the technical problem over its entire scope and thus the subject-matter of **claim 10-F** does not involve an inventive step over the prior art (Art. 56 EPC). The present application does not meet the requirement of Art. 52(1) EPC.

6.9 For the same reasons as set under item 6.2 and 6.3, an inventive step can be acknowledged for the method as disclosed in **claims 10-I and 10-J**.

6.10 In view of the above, an inventive step can be acknowledged for the method as disclosed in **claims 11, 13 and 15**.

7 REMARK

Since it is an accepted principle in patent systems that two patents shall not be granted to the same applicant for the same invention (Article 125 EPC and Guidelines G-IV 5.4), different applications having the same priority date and/ or filing dates may not claim the same subject-matter (Guidelines C-VI, 9.1.6). Thus, claims of applications from the same applicant need to clearly claim different subject-matter and simple rewording of the same technical features

should be strictly avoided. Since the claimed molecules, kits and methods are identical to that one claimed in the earlier application EP-06 751 097.9, the Applicant is kindly requested to delete the claims 6, 7, 8, 9, 10, 11, 12, 13, 15 or part thereof.

8 CONCLUDING REMARKS

Should the applicant enter the examination phase, the following would applied:

- it is not at present apparent which part of the application could serve as a basis for a new, allowable claim. Should the applicant nevertheless regard some particular matter as patentable, an independent claim should be filed taking account of Rule 43(1) EPC. The applicant should also indicate in the letter of reply the difference of the subject-matter of the new claim vis-à-vis the state of the art and the significance thereof.

- care would have to be taken during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Art. 123(2) EPC).

- to meet the requirements of Rule 42(1)(b) EPC, the documents D1-D12 would have to be identified in the description and the relevant background art disclosed therein would have to be briefly discussed.

- the amendments would have to be filed taking into account the requirements of R. 50 EPC. The passages in the application as originally files which support the amendments should be clearly indicated (Art. 123(2) EPC). To expedite the procedure, the Applicant is kindly asked to clearly indicate where the amendments have been made, possibly by filing a copy of the original sheets with the hand-written amendments.

- if amended claims are submitted, attention should be taken to the requirement of R. 43(2) EPC with respect to the number of independent claims.

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
02.10.15

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Communication

The extended European search report is enclosed.

The extended European search report includes, pursuant to Rule 62 EPC, the European search report (R. 61 EPC) or the partial European search report/ declaration of no search (R. 63 EPC) and the European search opinion.

Copies of documents cited in the European search report are attached.

☑ 0 additional set(s) of copies of such documents is (are) enclosed as well.

The following have been approved:

☑ Abstract ☑ Title

☐ The Abstract was modified and the definitive text is attached to this communication.

The following figure(s) will be published together with the abstract:

Refund of search fee
If applicable under Article 9 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.

Should you wish to further prosecute this application in the examination phase, your attention is drawn to the provisions of Rule 70a EPC. An invitation to respond to the extended European search report will be issued once the date of publication of the European search report has been mentioned in the European Patent Bulletin (R. 69(1), R. 70(2) EPC).

Europäisches Patentamt • European Patent Office • Office européen des brevets

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date: 07.10.15

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404

Applicant/Proprietor: Dow AgroSciences, LLC, et al

Refund of fees

The following fee(s) was (were) paid in respect of the application 15168051.9:

Fee	Reference	Voucher No	Date	Currency	Amount
Search fee	002	00840400	18.05.15	EUR	1 285,00

The fee(s) was (were) paid by J.A. Kemp.

According to the present state of the file the refund will be made by:

CREDITING THE DEPOSIT ACCOUNT 28050038 J.A. Kemp.

Amount refundable:	Reference	Currency	Amount	Voucher No
	002	EUR	1.079,40	01187476

Reason for refund: Full use of earlier search report with written opinion drawn up by the EPO (84 % of fee paid for current search refunded).

The Authorising Officer
Langer
(49)(89)23998205

Europäisches Patentamt • European Patent Office • Office européen des brevets

EPO Form 2907 04.14

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
14.10.15

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Notification of European publication number and information on the application of Article 67(3) EPC

You are hereby informed that the technical preparations for the publication of the above-mentioned European patent application have been completed.

The application will be published on 11.11.15 with the European search report.
The publication number is: 2942402.
The publication will be mentioned in European Patent Bulletin number 2015/46.
(**http://bulletin.european-patent-office.org**).

The provisional protection under Article 67(1) and (2) EPC in the individual contracting states becomes effective only when the conditions referred to in Article 67(3) EPC have been fulfilled. For further information, also with respect to extension states, please refer to the EPO brochure "National Law relating to the EPC" (**http://www.epo.org/patents/law/legal-texts/national-law-epc.html**).

The title of the invention in the three official languages of the European Patent Office is worded as follows:

MAISEREIGNIS TC1507 UND VERFAHREN ZUM NACHWEIS DAVON
CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF
ÉVÉNEMENT DE MAÏS TC1507 ET PROCÉDÉS DE DÉTECTION DE CELUI-CI

In all future communications to the EPO, please quote the application number as indicated above, i.e. including the final four figures (which identify the Directorate responsible for the subsequent procedure).

Remark
The publication can be downloaded, free of charge, from the EPO publication server **https://data.epo.org/publication-server/** or can be ordered from the Vienna sub-office upon payment of a fee (see Decision of the President of the EPO dated 12 July 2007 concerning the form of publication of European patent applications, European search reports and European patent specifications, Special edition No. 3, OJ EPO 2007, 97).

Note concerning renewal fees
Under Article 86 EPC renewal fees shall be paid to the EPO for the European patent application. These fees shall be due in respect of the third and each subsequent year, calculated from the date of filing. Due account should be taken that the EPO will not send invoices or reminders to pay any fees in due time.

Accelerated prosecution of European patent applications
Applicants wishing to accelerate proceedings before the EPO may file a request according to the programme for accelerated prosecution of European patent applications - "PACE" (OJ EPO 2010, 352). EPO Form 1005 is available on the EPO website (www.epo.org) for this purpose.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
16.11.15

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Communication pursuant to Rule 69 EPC - reminder concerning payment of the designation fee (Art. 79(2) EPC) and of the examination fee (Art. 94(1) EPC) - and invitation pursuant to Rule 70a(1) EPC

The date on which the European Patent Bulletin mentions the publication of the European search report for the above-mentioned European patent application is: 11.11.15.

Your attention is drawn to Article 79(2) and Rule 39(1) EPC as well as Article 94(1), Rule 70(1) and Rule 70a(1) EPC, according to which within **six months** of the above-mentioned publication date of the search report

- the designation fee must be paid,
- the request for examination must be filed and the examination fee must be paid,
- comments on the objections raised in the opinion accompanying the European search report and/or any amendments to the description, claims and drawings correcting any deficiencies noted in the opinion must be filed (R. 70a(1), 137(2) EPC; Guidelines for Examination in the EPO, B-XI, 8).

1. Payment of the designation fee and of the examination fee

The current designation fee for one or more Contracting States designated is: EUR 580,00

The current examination fee is: EUR 1620,00

If the written request for examination is filed in an admissible non-EPO language in accordance with Article 14(4) EPC, a reduction of the examination fee according to Rule 6(3) EPC applies.

For European patent applications filed on or after 01.04.2014, the reduction applies only if the applicant(s) has (have) declared to be an entity or a natural person within the meaning of Rule 6(4) EPC (Decision of the Administrative Council of 13 December 2013, OJ EPO 2014, A4).

If the designation fee and the examination fee are not paid within the period laid down in Rule 39(1) and Rule 70(1) EPC, the application **will be deemed to be withdrawn** (R. 39(2) and Art. 94(2) EPC).

Any extension or validation fees are also payable within the above-mentioned period.

Registered letter
EPO Form 1081 06.15 (NFS/RTB/ESOP01=N)

2. Comments on the opinion accompanying the European search report and filing of amendments

Should the reply to the invitation pursuant to Rule 70a(1) EPC be filed in an admissible non-EPO language, a translation is to be submitted within one month of its filing (R. 6(2) EPC).

If filing amendments, you must identify them and indicate the basis for them in the application as filed. Failure to meet either requirement may lead to a communication from the Examining Division requesting that you correct this deficiency (R. 137(4) EPC).

Should you not comply with this invitation within the time limit, the application **will be deemed to be withdrawn** in accordance with Rule 70a(3) EPC.

Note to users of the automatic debiting procedure
Unless the EPO receives prior instructions to the contrary, the examination fee and the designation fee will be debited on the last day of the period for payment. For further details see the Arrangements for the automatic debiting procedure, supplementary publication 3 - OJ EPO 2015.

Important information concerning fee amounts
Following any amendment to the Rules relating to Fees, the amount(s) mentioned in this communication may be different from the amount(s) **actually due on the date of payment.** The latest version of the Schedule of fees and expenses, published as a Supplement to the Official Journal of the EPO, is also available on the EPO website (**www.epo.org**) and can be found under **www.epo.org/schedule-of-fees**, which allows the viewing, downloading and searching for individual fee amounts, both current and previous.
Please note that procedural fees are usually adjusted every two years, on even years, with effect from 1 April.

Payments by cheque delivered or sent direct to the EPO are no longer accepted as from 1 April 2008 (see OJ EPO 2007, 626).

Note concerning renewal fees
Under Article 86 EPC renewal fees shall be paid to the EPO for the European patent application. These fees shall be due in respect of the third and each subsequent year, calculated from the date of filing. Due account should be taken that the EPO will not send invoices or reminders to pay any fees in due time.

Receiving Section



Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

Mr Andrew BENTHAM
J A Kemp
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
NL-2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Amended claims (clean copy)	Amended claims clean May16 N96314A-EP AXB KKT.pdf	CLMS-1.pdf
2	Amended claims with annotations	Amended Tracked claims May16 N96314A-EP AXB KKT.pdf	CLMS-HWA-1.pdf
3	Letter relating to the search and examination procedure	Response to SO N96314A-EP AXB KKT.pdf	EPLETT-1.pdf

	Fees	Factor applied	Fee schedule	Amount to be paid
15-1	005e Designation fee - For all contracting States designated for applications filed on/after 01.04.2009	1	585.00	585.00
15-2	006 Examination fee - For applications filed on/after 01.07.2005	1	1 635.00	1 635.00
	Total:		**EUR**	**2 220.00**

	Payment	
1	Mode of payment	Debit from deposit account
	Currency:	EUR
	The European Patent Office is hereby authorised, to debit from the deposit account with the EPO any fees and costs indicated on the fees page.	
	Deposit account number:	28050038
	Account holder:	J A Kemp
2	Refund/Reimburserment	
	Reimbursement (if any) to be made to EPO deposit account:	28050038
	Account holder:	J A Kemp

Signatures

Place:	
Date:	**11 May 2016**
Signed by:	**Andrew Bentham 23464**
Representative name:	**Andrew BENTHAM**
Capacity:	**(Representative)**

J A · K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

11 May 2016

Dear Sirs

European Patent Application No. 15168051.9-1404
DOW AGROSCIENCES LLC *et al.*
Our Ref: N96314A-EP AXB/KKT/mqr

I request substantive examination of this Divisional application. I also request that the following fees be deducted from deposit account No. 2805.0038 in the name of J A Kemp:

- the fee for substantive examination (EUR 1635); and
- the designation fee (EUR 585, for all available states).

A fee voucher showing these payments is attached. I request that the EPO takes any shortfall in these fees from the same deposit account.

In response to the Communication dated 14 October 2015, on behalf of the Applicants', my comments are set out below.

Amendments and basis thereof

Please find attached new claims page 53 to replace the claims presently on file. Also attached is a track-changes version of the claims currently on file showing the changes being made.

- Previous claim 1, 6 to 13 and 15 have been cancelled to advance prosecution.

- Previous claim 5 (new claim 4) has been re-formulated such that it is now directed to an "*insect resistant corn plant obtainable by*" the recited process.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • MUNICH
14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**
A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

- For the purposes of clarity and consistency, previous claim 14 (new claim 5) has been amended to specify that the TC1507 event is "*of SEQ ID NO: 24*". Basis for this amendment can be found in Table 1 of both the parent and divisional specifications as filed (see pages 30 to 31).

The remaining claims have been re-numbered and re-appended accordingly.

Comments

In view of the cancellation of the claims discussed above, the Examiner's clarity objections in Item 3 of the Search Opinion no longer apply. Accordingly, the claims meet the requirements of Article 84 EPC.

In view of the amendment made to previous claims 5 (see new claim 4), the Examiner's objection under Article 53(b) EPC no longer applies. As stated at the end of section G-II, 5.4.2 of the Guidelines: "*it is of no relevance that the protection conferred* [a] *product claim encompasses the generation of* [a] *claimed product by means of an essentially biological process*". Thus, in line with Decisions G2/12 and G2/13 of the EPO's Enlarged Board of Appeal, a claim directed to a product obtainable by a process, even if such process contains an essentially biological step, is not, as such, excluded from patentability.

It is noted that in Items 5 and 6 of the Search Opinion, the Examiner has acknowledged both novelty and inventive step for presently claimed subject-matter.

As requested by the Examiner, previous claims 6 to 13 and 15 have been cancelled. Thus, the subject-matter now claimed is clearly distinct from the subject-matter claimed in the granted European case 04751097.9, and no issues of double patenting arise.

I look forward to hearing from the Examining Division in due course. If there are any minor points that could usefully be discussed by telephone, the Examiner is welcome to give me a call. If further substantive issues arise, it is requested that another Examination Report be issued. If the Examining Division is minded to refuse the Application without issuing another Examination Report, Oral proceedings are requested as a precaution.

Yours faithfully

Electronically Signed
A BENTHAM

CLAIMS

~~1. An insect resistant corn plant or parts thereof, or corn seed, comprising event TC1507.~~

1~~2~~. An insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

2~~3~~. The plant or seed of claim~~s~~ 1 ~~or 2~~, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising event TC1507 or SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

3~~4~~. An insect resistant corn plant or parts thereof, or a descent plant of said corn plant, or corn seed, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the genome of the plant or seed.

4~~5~~. ~~A method of producing a~~An insect resistant corn plant obtainable by ~~comprising~~ breeding with a plant of claim 3~~4~~, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

6. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

7. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising at least a first primer, which recognizes a sequence within SEQ ID NO:21 or within SEQ ID NO:22; preferably

(a) further comprising at least a second primer which recognizes:

(i) a sequence within SEQ ID NO:25; more preferably wherein said at least first and second primers comprises the sequence of SEQ ID NO:1 and SEQ ID NO:2 respectively, or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively; or

(ii) a sequence within SEQ ID: 22; more preferably wherein said at least first and second primers comprises the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively, or the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively; or

(b) wherein the first primer recognizes a sequence within SEQ ID NO:21 or 22.

8. A DNA detection kit:

A. specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 26 or 27; or

B. comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny; or

C. comprising:

(a) a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the

group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(b) a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion, optionally wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

9. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with:

(a) SEQ ID NO:21 and SEQ ID NO:25, contiguous therewith; or

(b) SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

10. A method:

A. for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22; preferably

(a) further comprising amplifying a DNA fragment from a nucleic acid present in said biological sample using a polymerase chain reaction with at least two primers, wherein said first primer recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, and a second primer recognizes a sequence within SEQ ID NO:22 or SEQ ID NO: 25; more preferably wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25; wherein said first and second primers comprise:

(i) the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively, comprising amplifying a fragment of about 912 bp using TC1507 PCR identification protocol; or

(ii) the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively, comprising amplifying a fragment of about 844 bp using TC1507 PCR identification protocol; or

(b) wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22; more preferably wherein said first and second primers comprise:

(i) the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively, comprising amplifying a fragment of about 342 bp using TC1507 PCR identification protocol; or

(ii) the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol; or

B. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(a) providing DNA primer molecules SEQ ID NO:1 and SEQ ID NO:2 ;

(a) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

C. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:2 and SEQ ID NO:23 ;

(c) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

D. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(a) providing DNA primer molecules SEQ ID NO:3 and SEQ ID NO:5 ;

(a) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

E. of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:4 and SEQ ID NO:5 ;

(b) providing DNA amplification reaction conditions;

(d) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(e) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507; or

F. of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:

(a) contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;

(b) subjecting the sample and probe to stringent hybridization conditions; and

(c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event; or

G. for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample; or

H. for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot; or

I. of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is on opposite sides of a sequence diagnostic for said TC1507 event insertion;

(a) contacting a sample of said corn tissue with said primer pair;

(a) performing DNA amplification and analyzing for an amplicons, optionally wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; or

J. of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(a) subjecting said sample and probe to stringent hybridization conditions; and

(a) analyzing for hybridization of the probe.

11. An isolated DNA molecule comprising:

(a) the amplicon produced by the method of claim 10B;

(b) the amplicon produced by the method of claim 10C;

(c) the amplicon produced by the method of claim 10D; or

(d) the amplicon produced by the method of claim 10E.

12. An isolated:

A. DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 or its complement; optionally further comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement;

B. DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; or

C. DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

13. A pair of:

A. DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:26 or 27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof; or

B. isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce

~~an amplicon diagnostic for event TC1507; optionally wherein each is chosen from SEQ ID NO:26 or 27.~~

5~~14~~. A kit for identifying event TC1507 of SEQ ID NO: 24 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

~~15. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.~~

CLAIMS

1. An insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

2. The plant or seed of claim 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising event TC1507 or SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

3. An insect resistant corn plant or parts thereof, or a descent plant of said corn plant, or corn seed, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the genome of the plant or seed.

4. An insect resistant corn plant obtainable by breeding with a plant of claim 3, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

5. A kit for identifying event TC1507 of SEQ ID NO: 24 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	4332452
Application number	EP15168051.9
Date of receipt	11 May 2016
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (2 p.) CLMS-HWA-1.pdf\Amended Tracked claims May16 N96314A-EP AXB KKT.pdf (8 p.) ep-sfd-request.xml CLMS-1.pdf\Amended claims clean May16 N96314A-EP AXB KKT.pdf (1 p.) EPLETT-1.pdf\Response to SO N96314A-EP AXB KKT.pdf (2 p.)
Submitted by	CN=Andrew Bentham 23464
Method of submission	Online
Date and time receipt generated	11 May 2016, 12:52 (CEST)
Message Digest	1E:F8:E8:1D:2F:29:A1:CC:53:5F:AC:D6:57:7B:23:C0:6A:A8:77:C2

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
30.08.16

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Expected start of examination

Please be informed that according to current work planning, substantive examination of the above-mentioned application is expected to begin on or after 31.10.16

If the European patent application is withdrawn, refused or deemed to be withdrawn before substantive examination has begun, the examination fee is refunded at a rate of 100% (Article 11(a) RFees as amended with effect from 1 July 2016).

For the Examining Division

Europäisches Patentamt • European Patent Office • Office européen des brevets •

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
24.01.2017

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404 / 2942402

Applicant/Proprietor
Dow AgroSciences, LLC, et al

With regard to examination proceedings in respect of the above-mentioned European patent application please be advised of the following:

According to the Notice from the European Patent Office dated 24 November 2016 concerning the staying of proceedings due to the Commission Notice on certain articles of the Directive 98/44/EC on the legal protection of biotechnological inventions, the President of the EPO has decided to stay examining and opposition proceedings which are pending before the EPO and in which the decision depends entirely on the patentability of a plant or animal obtained by an essentially biological process (cf. Notice from the EPO dated 24 November 216, OJ EPO A104).

The subject-matter of the invention/patent is a plant obtained by an essentially biological process for the production of plants.

Hence the proceedings in the present case are stayed until the impact of the Commission notice on the EPO's practice has been clarified.

Once the impact of the Commission notice on the EPO's practice will have been clarified, examination proceedings will be resumed.

Attention is drawn to the possibility of refund of the examination fee under Article 11(a) and (b) Rfees (cf. OJ EPO 2016, A49).]

The Examining Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

Registered letter
EPO Form 2901 12.07 (19/01/17)

EP50828

J A • K E M P

FAX

Sent to Fax No:+49 89 2399 4465
2 Pages

Claudia Weber
The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

ATTENTION: CLAUDIA WEBER, LEGAL DIVISION

06 June 2017

PLEASE SEE ACCOMPANYING PAGES

Please acknowledge safe and complete receipt of this transmission

SIGNED DATE:

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 F +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**
A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

Duration: 06.06.2017 15:18:51 - 06.06.2017 15:19:21. This page 1 of 2 was completed at 06.06.2017 15:19:01
Received at the EPO on Jun 06, 2017 15:19:21. Page 1 of 2

J A · KEMP

BY FAX

Claudia Weber
The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

ATTENTION: CLAUDIA WEBER, LEGAL DIVISION

06 June 2017

Dear Ms Weber

Registration of J A Kemp as an association of professional representatives
- **Association No. 811**

J A Kemp has been registered as an association of professional representatives under the no. 811 with effect from 24 May 2017.

I request that all the live European cases on the EPO's records, in which the professional representative is an EP attorney from J A Kemp, be transferred to the newly registered association (J A Kemp - Association No. 811). A listing of the current members of the association was sent to me by the Legal and Unitary Patent Division by letter of 30 May 2017. If you need a copy of that letter please let me know.

I confirm that this is to include both pending European patent application and oppositions.

My understanding is that this transfer would normally trigger the issue of a hard copy EPO form on each affected case. We do not wish to receive an individual notification on each case affected. Please would you suppress the issue of those individual notification and instead simply send us one single listing of the cases affected.

We currently receive Communications on our EP cases via the EPO's Mailbox service. We wish this to continue.

Yours faithfully

Electronically Signed
NIGEL PRICE
J A KEMP

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**
A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

J A • KEMP

BY FAX

Claudia Weber
The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

EPO - Munich
11
07 Juni 2017

ATTENTION: CLAUDIA WEBER, LEGAL DIVISION

06 June 2017

Dear Ms Weber

Registration of J A Kemp as an association of professional representatives
- **Association No. 811**

J A Kemp has been registered as an association of professional representatives under the no. 811 with effect from 24 May 2017.

I request that all the live European cases on the EPO's records, in which the professional representative is an EP attorney from J A Kemp, be transferred to the newly registered association (J A Kemp - Association No. 811). A listing of the current members of the association was sent to me by the Legal and Unitary Patent Division by letter of 30 May 2017. If you need a copy of that letter please let me know.

I confirm that this is to include both pending European patent application and oppositions.

My understanding is that this transfer would normally trigger the issue of a hard copy EPO form on each affected case. We do not wish to receive an individual notification on each case affected. Please would you suppress the issue of those individual notification and instead simply send us one single listing of the cases affected.

We currently receive Communications on our EP cases via the EPO's Mailbox service. We wish this to continue.

Yours faithfully

~~Electronically Signed~~
NIGEL PRICE
J A KEMP

7.6.17.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**
A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
12.06.17

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1404 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Communication of amended entries concerning the representative (R. 143(1)(h) EPC)

As requested, for the above-mentioned European patent application / European patent the entries concerning the representative have been amended as follows:

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
GB

The amendment will be recorded in the Register of European Patents.

For the Examining Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

Application No.: 15168051.9

Substantive examination has started on 29.01.18 (see Notice from the European Patent Office dated 29.01.2013, OJ EPO 2013, 153).

For the Examining Division

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME-UNI

Formalities Officer
Name: Wezyk, Magdalena
Tel: +49 89 2399 - 8315
or call
+31 (0)70 340 45 00

Substantive Examiner
Name: Helliot, Bertrand
Tel: +49 89 2399 - 7793

Application No.	Ref.	Date
15 168 051.9 - 1118	N96314A-EP	05.02.2018
Applicant: Dow AgroSciences, LLC, et al		

Communication pursuant to Article 94(3) EPC

The examination of the above-identified application has revealed that it does not meet the requirements of the European Patent Convention for the reasons enclosed herewith. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to correct the indicated deficiencies within a period

of 4 months

from the notification of this communication, this period being computed in accordance with Rules 126(2) and 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

If filing amendments, you must identify them and indicate the basis for them in the application as filed. Failure to meet either requirement may lead to a communication from the Examining Division requesting that you correct this deficiency (R. 137(4) EPC).

In case you withdraw the application within the above-mentioned period, the examination fee will be refunded at a rate of 50% (Art. 11 (b) RFees).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

Registered letter

Helliot, Bertrand
Primary Examiner
For the Examining Division

Enclosure(s): 5 page/s reasons (Form 2906)

Registered letter
EPO Form 2001 11.16TRI

The examination is being carried out on the following application documents

Description, Pages

1-52 as originally filed

Sequence listings, SEQ ID NO

1-57 as originally filed

Claims, Numbers

1-5 received on 18-05-2016 with letter of 18-05-2016

Drawings, Sheets

1/1 as originally filed

1 INTRODUCTION

1.1 When drafting this communication, the Examining Division has taken the arguments provided by the Applicant in his reply of May, 15th 2015, into consideration.

2 ART. 123(2) EPC

The amendments filed with the letter dated May, 15th 2015, do not appear to introduce subject-matter which extends beyond the content of the application as filed, and therefore meet the requirement of Article 123(2) EPC.

3 Art. 53(b) EPC

The Applicant is kindly stressed that according to Article 53(b) EPC, plant or animal varieties as well as essentially biological processes for the production of plants or animals are excluded from patentability. The expressions "varieties" and "essentially biological process" were interpreted by the Enlarged Board of Appeals in their decisions G 1/98 and G 2/07, respectively. New Rule 28(2) EPC specifies that plants or animals which are exclusively obtained by means of an essentially biological process are also excluded from patentability under Article 53(b) EPC.

However, transgenic plants or animals are made by transforming a plant or parts thereof, or a fertilised animal embryo or stem cell in the case of animals, with a nucleic acid of interest. Transformation is a technical process, and **transgenic plants or animals are thus patentable**.
Descendants of the transformed plants or animals usually are obtained by crossing and selection, i.e. essentially biological processes. However, transgenic descendants are technically characterised by the transgene they carry. The technical effect of these transgenic plants or animals is the result of a technical process (the transformation step), and this technical step is crucial to obtain the product. Transgenic plants or animals cannot be obtained **exclusively** by means of an essentially biological process. **As a consequence, transgenic plants or animals are patentable, regardless of their generation**.

4 CLARITY (Art. 84 EPC)

4.1 The Examining Division is still of the opinion that the subject-matter of independent **claim 2,** which relates to an insect resistant corn line comprising event TC1507, lacks clarity in the sense of Article 84 EPC because the term TC1507 is an arbitrary internal designation which gives a vague and unclear definition.

5 NOVELTY (Art. 52(1) and Art. 54(1)(2) EPC)

5.1 D1 relates to the transgenic maize line TC1507. However, D1 does not discloses the sequence of the regions surrounding the transgenic event.

However, in such a case, the Enlarged Board of Appeal held in the decision G1/92 that the chemical composition of a product forms part of the state of the art when the product as such is available to the public and can be analysed and reproduced by the skilled person, irrespective of whether or not particular reasons can be identified for analysing the composition.

With respect to the parental application (EP-04751097.9), the Applicant provided evidence (see letter dated 16.10.2014) showing that the maize TC1507 was not available to the public for scientific purposes. The skilled person was thus not in the position to analyse and reproduce said transgenic maize. Accordingly, the subject-matter of independent **claim 1** is considered novel (Art. 54(1) and (2) EPC).

5.2 The same applies to **claims 3 and 4** (Art. 54(1)(2) EPC).

5.3 The same applies to the kit of **claim 5** (Art. 54(1)(2) EPC).

6 INVENTIVE STEP (Art. 52(1) and 56 EPC)

6.1 D1 relates to the transgenic maize line TC1507. The maize TC1507 was not available to the public for scientific purpose as stated by the Applicant in his letter dated 16-10-2014 (see ealier application). It was thus not possible for the skilled person to know the sequence corresponding to the event TC1507 and to the region surrounding the transgenic event TC1507. Accordingly, an inventive step can be acknowledged for the insect resistant corn plant or parts thereof as disclosed in **claim 1** (Art. 56 EPC).

6.2 An inventive step can be acknowledged for the kit of **claim 5** (Art. 56 EPC).

6.3 Present **claim 3** relates to an insect resistant corn plant or parts thereof, or a descent plant of said corn plant, or corn seed, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the genome of the plant or seed.

In accordance with the case law, there can only be an invention if the application makes it at least plausible that its teaching indeed solves the problem it purports to solve.

In the present case, the Examining Division is of the opinion that DNA molecules comprising one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof cannot confer the insect resistance to the corn plant, a part thereof, descent plant or seed.

Accordingly, the Examining Division is of the opinion that the present **claim 3** does not solve the technical problem it purported to solve. Therefore, the subject-matter of **claim 3** does not involve an inventive step over the prior art (Art. 56 EPC) and the present application does not meet the requirement of Art. 52(1) EPC.

6.4 The same applies to **claim 4** (Art. 56 EPC).

7 REMARK

Since it is an accepted principle in patent systems that two patents shall not be granted to the same applicant for the same invention (Article 125 EPC and Guidelines G-IV 5.4), different applications having the same priority date and/or filing dates may not claim the same subject-matter (Guidelines C-IX, 1.6). Thus, claims of applications from the same applicant need to clearly claim different subject-matter and simple rewording of the same technical features should be strictly avoided.

Since the claimed kit is identical to that one claimed in the earlier application, the Applicant is kindly requested to delete **claim 5**.

8 CONCLUDING REMARKS

8.1 The applicant is requested to file new claims which take account of the above comments.

8.2 Care would have to be taken during revision, especially of the introductory portion and any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed (Art. 123(2) EPC).

8.3 The amendments would have to be filed taking into account the requirements of R. 50 EPC. The passages in the application as originally files which support the amendments should be clearly indicated (Art. 123(2) EPC). To expedite the procedure, the Applicant is kindly asked to clearly indicate where the amendments have been made, possibly by filing a copy of the original sheets with the hand-written amendments.

8.4 If amended claims are submitted, attention should be taken to the requirement of R. 43(2) EPC with respect to the number of independent claims.

J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

1 June 2018

Dear Sirs

European Patent Application No. 15168051.9
DOW AGROSCIENCES LLC *et al.*
Our Ref: N96314A-EP AXB/KKT/mqr

In response to the Examination Report dated 5 February 2018, please replace the claims at present on file with the new claims provided. Please also replace the description currently on file with the new description provided.

A marked-up version of the claims and description, showing the amendments being made, is also provided for the Examiner's reference.

Amendments

Merely to advance prosecution, previous claims 3 to 5 have been deleted.

In addition, the reference to "*event TC1507*" in claim 2 has been cancelled.

The description has been amended for conformity with the claims. In particular, counterparts to the claims have been added at page 3.

In addition, the title at the top of page 1, statements of incorporation by reference, and statements suggesting that the scope of the invention may extend beyond the scope of the claims, have all been deleted.

Comments

The Applicants are pleased to see that the Examining Division acknowledges that claims 1 and 2 are essentially allowable. The Examining Division's remaining objections are discussed below, with reference to the numbering used in the Examination Report.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • PARIS • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

4 Merely to advance prosecution, the reference to "*event TC1507*" in claim 2 has been cancelled. Therefore, the TC1507 event has been defined in the claims by reference solely to SEQ ID NO: 24, which necessarily defines the event TC1507, as SEQ ID NO: 24 comprises the insert and the 5' and the 3' flanking regions. Accordingly, the claims meet the requirements of Article 84 EPC.

5/6 In view of the cancellation of claims 3 and 4, the Examining Division's inventive step objection has been overcome. Accordingly, the amended claims meet the requirements of Article 54 and 56 EPC.

7 In view of the deletion of claim 5, no double patenting exists between the claims of the present Divisional application and the claims of the Parent case EP1620571.

8.4 In view of the cancellation of claims 3 to 5, the claims do not contain multiple independent claims in the same claim category (Rule 43(2) EPC).

Concluding Remarks

The Application is now believed to be in an allowable form and the Examiner is asked to reconsider it favourably. If, however, there are any queries outstanding, please issue a further Examination Report or telephone the undersigned. Only as a precaution in case the Examiner is minded to reject the Application without issuing first a further Examination Report, I request Oral Proceedings.

I look forward to hearing from the Examiner in due course.

Yours faithfully

Electronically Signed
A BENTHAM
ASSOCIATION NO. 811

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Reply to examination report	Response N96314A-EP AXB KKT.PDF	EXRE3-1.PDF
2	Amended claims (clean copy)	Amended claims (clean) Jun18 N96314A-EP AXB KKT.PDF	CLMS-1.PDF
3	Amended claims with annotations	Amended claims (tracked) Jun18 N96314A-EP AXB KKT.pdf	CLMS-HWA-1.pdf
4	Amended description (clean copy)	Amended descr (clean) Jun18 N96314A-EP AXB KKT.PDF	DESC-1.PDF
5	Amended description with annotations	Amended desc (tracked) Jun18 N96314A-EP AXB KKT.pdf	DESC-HWA-1.pdf

	Payment	
1	Method of payment	Not specified

Signatures

Place:	**London, GB**
Date:	**01 June 2018**
Signed by:	**Kapil Tuladhar 55592**
Association:	**J A Kemp**
Representative name:	**Kapil TULADHAR**
Capacity:	**(Representative)**

FIELD OF THE INVENTION

The present invention relates to the field of plant molecular biology, specifically the invention relates to a DNA construct for conferring insect resistance to a plant. The invention more specifically relates to an insect resistant corn plant TC1507.

BACKGROUND OF THE INVENTION

This invention relates to the insect resistant corn (*Zea mays*) plant TC1507, also referred to as maize line TC1507 or maize event TC1507.

Corn is an important crop and is a primary food source in many areas of the world. Damage caused by insect pests is a major factor in the loss of the world's corn crops, despite the use of protective measures such as chemical pesticides. In view of this, insect resistance has been genetically engineered into crops such as corn in order to control insect damage and to reduce the need for traditional chemical pesticides. One group of genes which have been utilized for the production of transgenic insect resistant crops are the delta-endotoxins from *Bacillus thuringiensis (B.t.)*. Delta-endotoxins have been successfully expressed in crop plants such as cotton, potatoes, rice, sunflower, as well as corn, and have proven to provide excellent control over insect pests. (Perlak, F.J *et al.* (1990) *Bio/Technology* 8, 939-943; Perlak, F.J. *et al.* (1993) *Plant Mol. Biol.* 22: 313-321; Fujimoto H. *et al.* (1993) *Bio/Technology* 11: 1151-1155; Tu *et al.* (2000) *Nature Biotechnology* 18:1101-1104; PCT publication number WO 01/13731; and Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, Fort Collins, CO).

The expression of foreign genes in plants is known to be influenced by their location in the plant genome, perhaps due to chromatin structure (e.g., heterochromatin) or the proximity of transcriptional regulatory elements (e.g., enhancers) close to the integration site (Weising *et al.*, *Ann. Rev. Genet* 22:421-477, 1988). At the same time the presence of the transgene at different locations in the genome will influence the overall phenotype of the plant in different ways. For this reason, it is often necessary to screen a large number of events in order to identify an event characterized by optimal expression of an introduced gene of interest. For example, it has been observed in plants and in other organisms that there may be a wide variation in levels of expression of an introduced gene among events. There may also be differences in spatial or temporal patterns of expression, for example, differences in the relative expression of a transgene in various plant tissues, that may not correspond to the patterns expected from transcriptional regulatory elements

present in the introduced gene construct. For this reason, it is common to produce hundreds to thousands of different events and screen those events for a single event that has desired transgene expression levels and patterns for commercial purposes. An event that has desired levels or patterns of transgene expression is useful for introgressing the transgene into other genetic backgrounds by sexual outcrossing using conventional breeding methods. Progeny of such crosses maintain the transgene expression characteristics of the original transformant. This strategy is used to ensure reliable gene expression in a number of varieties that are well adapted to local growing conditions.

It would be advantageous to be able to detect the presence of a particular event in order to determine whether progeny of a sexual cross contain a transgene of interest. In addition, a method for detecting a particular event would be helpful for complying with regulations requiring the pre-market approval and labeling of foods derived from recombinant crop plants, for example, or for use in environmental monitoring, monitoring traits in crops in the field, or monitoring products derived from a crop harvest, as well as for use in ensuring compliance of parties subject to regulatory or contractual terms.

It is possible to detect the presence of a transgene by any nucleic acid detection method known in the art including, but not limited to, the polymerase chain reaction (PCR) or DNA hybridization using nucleic acid probes. These detection methods generally focus on frequently used genetic elements, such as promoters, terminators, marker genes, etc., because for many DNA constructs, the coding region is interchangeable. As a result, such methods may not be useful for discriminating between different events, particularly those produced using the same DNA construct or very similar constructs unless the DNA sequence of the flanking DNA adjacent to the inserted heterologous DNA is known. For example, an event-specific PCR assay is described in U.S. Patent No. 6,395,485 for the detection of elite event GAT-ZM1. Accordingly, it would be desirable to have a simple and discriminative method for the identification of event TC1507.

SUMMARY OF THE INVENTION

This disclosure relates preferably to methods for producing and selecting an insect resistant monocot crop plant. More specifically, a DNA construct is provided that when expressed in plant cells and plants confers resistance to insects. Accordingly a DNA construct, capable of introduction into and replication in a host cell, is provided that when expressed in plant cells and plants confers insect resistance to the plant cells and plants. The DNA construct is comprised of a DNA molecule named PHI8999A and it includes

two transgene expression cassettes. The first expression cassette comprises a DNA molecule which includes the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689 and Christensen and Quail (1996) *Transgenic Res.* 5:213-218) operably connected to a DNA molecule encoding a *B.t.* δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350). The second transgene expression cassette of the DNA construct comprises a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59) operably connected to a DNA molecule encoding a phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene* 70: 25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59). Plants containing the DNA construct are also provided.

One aspect of the invention is an insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

Another aspect of the invention is the plant or seed of aspect 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

The foregoing and other aspects of the invention will become more apparent from the following detailed description and accompanying drawing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Linear map showing the transgenic insert PHI8999A, as well as the sequences flanking the transgenic insert.

DETAILED DESCRIPTION

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al.*, *Glossary of Genetics: Classical and Molecular, 5th edition,* Springer-Verlag; New York, 1991; and Lewin, *Genes V,* Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at 37 CFR 1.822 is used.

As used herein, the term "comprising" means "including but not limited to".

As used herein, the term "corn" means *Zea mays* or maize and includes all plant varieties that can be bred with corn, including wild maize species.

As used herein, the term "TC1507 specific" refers to a nucleotide sequence which is suitable for discriminatively identifying event TC1507 in plants, plant material, or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material.

As used herein, the terms "insect resistant" and "impacting insect pests" refers to effecting changes in insect feeding, growth, and/or behavior at any stage of development, including but not limited to: killing the insect; retarding growth; preventing reproductive capability; and the like.

As used herein, the terms "pesticidal activity" and "insecticidal activity" are used synonymously to refer to activity of an organism or a substance (such as, for example, a protein) that can be measured by numerous parameters including, but not limited to, pest mortality, pest weight loss, pest attraction, pest repellency, and other behavioral and physical changes of a pest after feeding on and/or exposure to the organism or substance for an appropriate length of time. For example "pesticidal proteins" are proteins that display pesticidal activity by themselves or in combination with other proteins.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. As used herein, the terms "encoding" or "encoded" when used in the context of a specified nucleic acid mean that the nucleic acid comprises the requisite

information to guide translation of the nucleotide sequence into a specified protein. The information by which a protein is encoded is specified by the use of codons. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid or may lack such intervening non-translated sequences (e.g., as in cDNA).

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. "Foreign" refers to material not normally found in the location of interest. Thus "foreign DNA" may comprise both recombinant DNA as well as newly introduced, rearranged DNA of the plant. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The site in the plant genome where a recombinant DNA has been inserted may be referred to as the "insertion site" or "target site".

As used herein, "insert DNA" refers to the heterologous DNA within the expression cassettes used to transform the plant material while "flanking DNA" can exist of either genomic DNA naturally present in an organism such as a plant, or foreign (heterologous) DNA introduced via the transformation process which is extraneous to the original insert DNA molecule, e.g. fragments associated with the transformation event. A "flanking region" or "flanking sequence" as used herein refers to a sequence of at least 20 base pair, preferably at least 50 base pair, and up to 5000 base pair which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the original foreign insert DNA molecule. Transformation procedures leading to random integration of the foreign DNA will result in transformants containing different flanking regions characteristic and unique for each transformant. When recombinant DNA is introduced into a plant through traditional crossing, its flanking

regions will generally not be changed. Transformants will also contain unique junctions between a piece of heterologous insert DNA and genomic DNA, or 2 pieces of genomic DNA, or 2 pieces of heterologous DNA. A "junction" is a point where 2 specific DNA fragments join. For example, a junction exists where insert DNA joins flanking DNA. A junction point also exists in a transformed organism where 2 DNA fragments join together in a manner that is modified from that found in the native organism. "Junction DNA" refers to DNA that comprises a junction point.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous nucleotide sequence can be from a species different from that from which the nucleotide sequence was derived, or, if from the same species, the promoter is not naturally found operably linked to the nucleotide sequence. A heterologous protein may originate from a foreign species, or, if from the same species, is substantially modified from its original form by deliberate human intervention.

"Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements are often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters that cause a nucleic acid fragment to be expressed in

most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect numerous parameters including, processing of the primary transcript to mRNA, mRNA stability and/or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol*. 3:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht *et al*. (1989) *Plant Cell* 1:671-680.

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide.

A DNA construct is an assembly of DNA molecules linked together that provide one or more expression cassettes. The DNA construct may be a plasmid that is enabled for self replication in a bacterial cell and contains various endonuclease enzyme restriction sites that are useful for introducing DNA molecules that provide functional genetic elements, i.e., promoters, introns, leaders, coding sequences, 3' termination regions, among others; or a DNA construct may be a linear assembly of DNA molecules, such as an expression cassette. The expression cassette contained within a DNA construct comprise the necessary genetic elements to provide transcription of a messenger RNA. The expression cassette can be designed to express in prokaryote cells or eukaryotic cells, most preferably in plant cells.

The DNA molecules are provided in expression cassettes for expression in an organism of interest. The cassette will include 5' and 3' regulatory sequences operably linked to a coding sequence. "Operably linked" means that the nucleic acid sequences

being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. Operably linked is intended to indicate a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes or multiple DNA constructs.

The expression cassette will include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region, a coding region, and a transcriptional and translational termination region functional in the organism serving as a host. The transcriptional initiation region (*i.e.*, the promoter) may be native or analogous, or foreign or heterologous to the host organism. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation.

It is to be understood that as used herein the term "transgenic" includes any cell, cell line, callus, tissue, plant part, or plant the genotype of which has been altered by the presence of a heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

A transgenic "event" is produced by transformation of plant cells with a heterologous DNA construct(s), including a nucleic acid expression cassette that comprises a transgene of interest, the regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and selection of a particular plant characterized by insertion into a particular genome location. An event is characterized phenotypically by the expression of the transgene. At the genetic level, an event is part of the genetic makeup of a plant. The term "event" also refers to progeny produced by a sexual outcross between the transformant and another variety that include the heterologous DNA. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed parent is present in the progeny of the cross at the

same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA and flanking sequence immediately adjacent to the inserted DNA that would be expected to be transferred to a progeny that receives inserted DNA including the transgene of interest as the result of a sexual cross of one parental line that includes the inserted DNA (e.g., the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA.

An insect resistant TC1507 corn plant can be bred by first sexually crossing a first parental corn plant consisting of a corn plant grown from the transgenic TC1507 corn plant and progeny thereof derived from transformation with the expression cassettes that confers insect resistance, and a second parental corn plant that lacks insect resistance, thereby producing a plurality of first progeny plants; and then selecting a first progeny plant that is resistant to insects; and selfing the first progeny plant, thereby producing a plurality of second progeny plants; and then selecting from the second progeny plants an insect resistant plant. These steps can further include the back-crossing of the first insect resistant progeny plant or the second insect resistant progeny plant to the second parental corn plant or a third parental corn plant, thereby producing a corn plant that is resistant to insects.

As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, and progeny of same. Parts of transgenic plants understood to be within the scope of the invention comprise, for example, plant cells, protoplasts, tissues, callus, embryos as well as flowers, stems, fruits, leaves, and roots originating in transgenic plants or their progeny previously transformed with a DNA molecule of the invention and therefore consisting at least in part of transgenic cells, are also an aspect of the present invention.

As used herein, the term "plant cell" includes, without limitation, seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants that can be used in the methods is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere *et al*. (1987) *Meth. Enzymol*. 143:277) and particle-accelerated

or "gene gun" transformation technology (Klein *et al.* (1987) *Nature* (London) 327:70-73; U.S. Patent No. 4,945,050). Additional transformation methods are disclosed below.

Thus, isolated polynucleotides can be incorporated into recombinant constructs, typically DNA constructs, which are capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels *et al.*, (1985; Supp. 1987) *Cloning Vectors: A Laboratory Manual*, Weissbach and Weissbach (1989) *Methods for Plant Molecular Biology*, (Academic Press, New York); and Flevin *et al.*, (1990) *Plant Molecular Biology Manual*, (Kluwer Academic Publishers). Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several references, e.g., Fehr, in *Breeding Methods for Cultivar Development*, Wilcos J. ed., American Society of Agronomy, Madison Wis. (1987).

A "probe" is an isolated nucleic acid to which is attached a conventional detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, or enzyme. Such a probe is complementary to a strand of a target nucleic acid, for example to a strand of isolated DNA from corn event TC1507 whether from a corn plant or from a sample that includes DNA from the event. Probes include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that bind specifically to a target DNA sequence and can be used to detect the presence of that target DNA sequence.

"Primers" are isolated nucleic acids that are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase. Primer pairs refer to their use for amplification of a target nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods. "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (see, U.S. Patent Nos. 4,683,195 and 4,800,159).

Probes and primers are of sufficient nucleotide length to bind to the target DNA sequence specifically in the hybridization conditions or reaction conditions determined by the operator. This length may be of any length that is of sufficient length to be useful in a detection method of choice. Generally, 11 nucleotides or more in length, preferably 18 nucleotides or more, and more preferably 22 nucleotides or more, are used. Such probes and primers hybridize specifically to a target sequence under high stringency hybridization conditions. Preferably, probes and primers have complete DNA sequence similarity of contiguous nucleotides with the target sequence, although probes differing from the target DNA sequence and that retain the ability to hybridize to target DNA sequences may be designed by conventional methods. Probes can be used as primers, but are generally designed to bind to the target DNA or RNA and not be used in an amplification process.

Specific primers can be used to amplify an integration fragment to produce an amplicon that can be used as a "specific probe" for identifying event TC1507 in biological samples. When the probe is hybridized with the nucleic acids of a biological sample under conditions which allow for the binding of the probe to the sample, this binding can be detected and thus allow for an indication of the presence of event TC1507 in the biological sample. Such identification of a bound probe has been described in the art. The specific probe is preferably a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the event and preferably also comprises a part of the foreign DNA contiguous therewith. Preferably the specific probe comprises a sequence of at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, and most preferably between 95 and 100% identical (or complementary) to a specific region of the event.

Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual, 2nd ed.*, vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*, Greene

Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992"); and Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as the PCR primer analysis tool in Vector NTI version 6 (Informax Inc., Bethesda MD); PrimerSelect (DNASTAR Inc., Madison, WI); and Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Additionally, the sequence can be visually scanned and primers manually identified using guidelines known to one of skill in the art.

A "kit" as used herein refers to a set of reagents for the purpose of performing the method, more particularly, the identification of the event TC1507 in biological samples. The kit can be used, and its components can be specifically adjusted, for purposes of quality control (e.g. purity of seed lots), detection of event TC1507 in plant material, or material comprising or derived from plant material, such as but not limited to food or feed products. "Plant material" as used herein refers to material which is obtained or derived from a plant.

Primers and probes based on the flanking DNA and insert sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed sequences by conventional methods, e.g., by re-cloning and sequencing such sequences. The nucleic acid probes and primers hybridize under stringent conditions to a target DNA sequence. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of DNA from a transgenic event in a sample. Nucleic acid molecules or fragments thereof are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another

under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, 1989, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, a Practical Approach,* IRL Press, Washington, D.C. (1985), departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

In hybridization reactions, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. The thermal melting point (Tm) is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. For DNA-DNA hybrids, the Tm can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: Tm = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. Tm is reduced by about 1°C for each 1% of mismatching; thus, Tm, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the Tm can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the Tm for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the Tm; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the Tm; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the Tm.

Using the equation, hybridization and wash compositions, and desired Tm, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a Tm of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in*

Molecular Biology, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al*. (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

As used herein, a substantially homologous sequence is a nucleic acid molecule that will specifically hybridize to the complement of the nucleic acid molecule to which it is being compared under high stringency conditions. Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2X SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of a destabilizing agent such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. A nucleic acid may specifically hybridize to one or more of the nucleic acid molecules unique to the TC1507 event or complements thereof or fragments of either under moderately stringent conditions.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local homology algorithm of Smith *et al*. (1981) *Adv. Appl. Math*. 2:482; the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol*. 48:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci*. 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0); the ALIGN PLUS program (version 3.0, copyright 1997); and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 10 (available from Accelrys, 9685 Scranton Road, San Diego, CA 92121, USA). Alignments using these programs can be performed using the default parameters.

The CLUSTAL program is well described by Higgins and Sharp, *Gene* 73: 237-244 (1988); Higgins and Sharp, *CABIOS* 5: 151-153 (1989); Corpet, *et al., Nucleic Acids Research* 16: 10881-90 (1988); Huang, *et al., Computer Applications in the Biosciences* 8: 155-65 (1992), and Pearson, *et al., Methods in Molecular Biology* 24: 307-331 (1994). The ALIGN and the ALIGN PLUS programs are based on the algorithm of Myers and Miller (1988) *supra.* The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra.* The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology*, Chapter 19, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). Alignment may also be performed manually by inspection.

To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) supra. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.hlm.nih.gov.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that

residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Regarding the amplification of a target nucleic acid sequence (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize only to the target nucleic-acid sequence to which a primer having the corresponding wild-type sequence (or its complement) would bind and preferably to produce a unique amplification product, the amplicon, in a DNA thermal amplification reaction.

The term "specific for (a target sequence)" indicates that a probe or primer hybridizes under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

As used herein, "amplified DNA" or "amplicon" refers to the product of nucleic acid amplification of a target nucleic acid sequence that is part of a nucleic acid template. For example, to determine whether a corn plant resulting from a sexual cross contains transgenic event genomic DNA from the corn plant of the present invention, DNA extracted from the corn plant tissue sample may be subjected to a nucleic acid amplification method using a DNA primer pair that includes a first primer derived from flanking sequence adjacent to the insertion site of inserted heterologous DNA, and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the event DNA. Alternatively, the second primer may be derived from the flanking sequence. The amplicon is of a length and has a sequence that is also diagnostic for the event. The amplicon may range in length from the combined length of the primer pairs plus one nucleotide base pair to any length of amplicon producible by a DNA amplification protocol. Alternatively, primer pairs can be derived from flanking sequence on both sides of the inserted DNA so as to produce an amplicon that includes the entire insert nucleotide sequence of the PHI8999A expression construct, see FIG. 1, approximately 6.2 Kb in size. A member of a primer pair derived from the flanking sequence may be located a distance from the inserted DNA sequence, this distance can range from one nucleotide base pair up to the limits of the amplification reaction, or about twenty thousand nucleotide base pairs. The use of the term "amplicon" specifically excludes primer dimers that may be formed in the DNA thermal amplification reaction.

Nucleic acid amplification can be accomplished by any of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described, inter alia, in U.S. Pat. Nos. 4,683,195 and 4,683,202 and in *PCR Protocols: A Guide to Methods and Applications,* ed. Innis *et al.,* Academic press, San Diego, 1990. PCR amplification methods have been developed to amplify up to 22 Kb of genomic DNA and up to 42 Kb of bacteriophage DNA (Cheng *et al., Proc. Natl. Acad. Sci. USA* 91:5695-5699, 1994). These methods as well as other methods known in the art of DNA amplification may be used. It is understood that a number of parameters in a specific PCR protocol may need to be adjusted to specific laboratory conditions and may be slightly modified and yet allow for the collection of similar results. These adjustments will be apparent to a person skilled in the art.

The amplicon produced by these methods may be detected by a plurality of techniques, including, but not limited to, Genetic Bit Analysis (Nikiforov, *et al. Nucleic*

Acid Res. 22:4167-4175, 1994) where a DNA oligonucleotide is designed which overlaps both the adjacent flanking DNA sequence and the inserted DNA sequence. The oligonucleotide is immobilized in wells of a microwell plate. Following PCR of the region of interest (using one primer in the inserted sequence and one in the adjacent flanking sequence) a single-stranded PCR product can be hybridized to the immobilized oligonucleotide and serve as a template for a single base extension reaction using a DNA polymerase and labeled ddNTPs specific for the expected next base. Readout may be fluorescent or ELISA-based. A signal indicates presence of the insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Another detection method is the Pyrosequencing technique as described by Winge (*Innov. Pharma. Tech.* 00: 18-24, 2000). In this method an oligonucleotide is designed that overlaps the adjacent DNA and insert DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted sequence and one in the flanking sequence) and incubated in the presence of a DNA polymerase, ATP, sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. dNTPs are added individually and the incorporation results in a light signal which is measured. A light signal indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single or multi-base extension.

Fluorescence Polarization as described by Chen *et al*., (*Genome Res*. 9:492-498, 1999) is a method that can be used. Using this method an oligonucleotide is designed which overlaps the flanking and inserted DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted DNA and one in the flanking DNA sequence) and incubated in the presence of a DNA polymerase and a fluorescent-labeled ddNTP. Single base extension results in incorporation of the ddNTP. Incorporation can be measured as a change in polarization using a fluorometer. A change in polarization indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Taqman® (PE Applied Biosystems, Foster City, Calif.) is described as a method of detecting and quantifying the presence of a DNA sequence and is fully understood in the instructions provided by the manufacturer. Briefly, a FRET oligonucleotide probe is designed which overlaps the flanking and insert DNA junction. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic

sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Hybridization of the FRET probe results in cleavage and release of the fluorescent moiety away from the quenching moiety on the FRET probe. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

Molecular Beacons have been described for use in sequence detection as described in Tyangi *et al.* (*Nature Biotech.* 14:303-308, 1996). Briefly, a FRET oligonucleotide probe is designed that overlaps the flanking and insert DNA junction. The unique structure of the FRET probe results in it containing secondary structure that keeps the fluorescent and quenching moieties in close proximity. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Following successful PCR amplification, hybridization of the FRET probe to the target sequence results in the removal of the probe secondary structure and spatial separation of the fluorescent and quenching moieties. A fluorescent signal results. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

A hybridization reaction using a probe specific to a sequence found within the amplicon is yet another method used to detect the amplicon produced by a PCR reaction.

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

EXAMPLES

Example 1. Transformation of Maize by Particle Bombardment and Regeneration of Transgenic Plants Containing the Cry1F Gene

A DNA molecule of 6.2 Kb, designated PHI8999A (see FIG. 1 and SEQ ID NO:25), which includes a first transgene expression cassette comprising the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) Plant Mol. Biol. 18:675-689 and Christensen and Quail (1996) Transgenic Res. 5:213-218) operably connected to a DNA molecule encoding a Bacillus thuringiensis δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350), and a second transgene expression cassette comprising a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) operably connected to a DNA molecule encoding the selectable marker, phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene 70*:25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) was used to transform maize embryo tissue.

B.t. Cry1F maize plants were obtained by microprojectile bombardment using the Biolistics® PDS-1000He particle gun manufactured by Bio-Rad, Hercules, CA; essentially as described by Klein *et al.* (1987) *Nature,* UK 327(6117):70-73. Immature embryos isolated from maize ears, harvested soon after pollination were cultured on callus initiation medium for several days. On the day of transformation, microscopic tungsten particles were coated with purified PHI8999A DNA (SEQ ID NO:25) and accelerated into the cultured embryos, where the insert DNA was incorporated into the cell chromosome. Only insert PHI8999A was used during transformation and no additional plasmid DNA was incorporated into the transformant. After bombardment, embryos were transferred to callus initiation medium containing glufosinate as the selection agent. Individual embryos were kept physically separate during culture, and the majority of explants died on the selective medium.

Those embryos that survived and produced healthy, glufosinate-resistant callus tissue were assigned unique identification codes representing putative transformation

events, and continually transferred to fresh selection medium. Plants were regenerated from tissue derived from each unique event and transferred to the greenhouse. Leaf samples were taken for molecular analysis to verify the presence of the transgene by PCR and to confirm expression of the Cry1F protein by ELISA. Plants were then subjected to a whole plant bioassay using European corn borer insects. Positive plants were crossed with inbred lines to obtain seed from the initial transformed plants. A number of lines were evaluated in the field. The TC1507 event was selected from a population of independent transgenic events based on a superior combination of characteristics, including insect resistance and agronomic performance (see Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, fort Collins, CO).

Example 2. Identification of nucleotides comprising the flanking sequence 5' to the transgenic insert DNA in *Bacillus thuringiensis* Cry1F maize line TC1507

To identify a DNA fragment that included sequence 5' to the PHI8999A insert in event TC1507, Spe I restriction enzyme fragments from event TC1507 genomic DNA were size selected on agarose gels, purified, and screened by Southern analysis to confirm hybridization to a Cry1F probe. Following confirmation of hybridization and fragment size, the fragments of interest were cloned into a pBluescript II SK (+)™ cloning vector to prepare an enriched size selected plasmid based genomic DNA library. A probe homologous to a portion of the Cry1F gene was used to screen the plasmid library for positive clones. A positive clone was identified, purified by additional screening, and confirmed to result in a positive signal when hybridized to the Cry1F probe. Nearly 3 Kb of the Spe I fragment contained in the isolated positive clone was sequenced using a primer walking approach. To initiate the first sequencing run, a primer that binds to a known sequence in the cloning vector DNA was designed to sequence a portion of the DNA of interest. A second sequencing run over the same region using another primer oriented in the reverse direction provided second strand coverage. Primer walking was accomplished by repeatedly using sequence data from previous runs to design new primers that were then used to extend the next round of sequencing further into the DNA of interest until the flanking sequence 5' to the inserted transgenic DNA in maize event TC1507 was obtained. Specific sequence information is provided in Example 4.

Example 3. Confirmation of flanking sequence 5' to the *B.t.* Cry1F maize line TC1507 insert

To confirm the 5' flanking sequence of the *B.t.* Cry1F maize line TC1507 insert, PCR primer pairs were designed to obtain overlapping PCR products extending from the 5' flanking region into the full-length PHI8999A transgenic insert. PCR products were successfully amplified from *B.t.* Cry1F maize line TC1507 genomic DNA, isolated, and sequenced for Region 1 through Region 6, shown in Table 1, and confirmed to match the previously determined sequence from the Spe I fragment, described in Example 2. However, the region from bp 2358 to bp 2829, immediately adjacent and 5' to the start of the full-length insert was recalcitrant to PCR amplification and appeared to be larger than the sequence obtained from the Spe I clone described above. The use of primer pairs flanking this region and the Advantage®-GC 2 Polymerase Mix (BD Biosciences Clontech, Palo Alto, CA) was successful in amplifying PCR products from *B.t.* Cry1F maize line TC1507 genomic DNA for sequencing. The amplification conditions used to produce amplicons with the Advantage®-GC 2 system are shown in Table 10. The DNA primer pairs used to confirm the sequence in the region from bp 2358 to 2829 are those listed in SEQ ID NO:1 and SEQ ID NO:2; and SEQ ID NO:2 and SEQ ID NO:23. Sequence from this region is described in Table 1 (Regions 7a, 7b, 7c, and 8).

Example 4. Event TC1507 5' flanking sequence. A description of each region is provided in Table 1.

Region 1 (SEQ ID NO: 28) Maize genomic (no significant homology)

```
   1 ACTAGTTTCC TAGCCCGCGT CGTGCCCCTA CCCCACCGAC
     GTTTATGGAA
  51 GGTGCCATTC CACGGTTCTT CGTGGCCGCC CCTAAGGATG
     TAAATGGTCG
 101 GTAAAATCCG GTAAATTTCC GGTACCGTTT ACCAGATTTT
     TCCAGCCGTT
 151 TTCGGATTTA TCGGGATATA CAGAAAACGA GACGGAAACG
     GAATAGGTTT
 201 TTTTTCGAAA ACGGTACGGT AAACGGTGAG ACAAACTTAC
     CGTCCGTTTT
 251 CGTATTTCTC GGGAAACTCT GGTATATTCC CGTATTTGTC
     CCGTATTTTC
 301 CCGACCCACG GACCTGCCAA TCAACCATCA GCCAGTCAGC
     CCATCCCCAC
 351 AGCTATGGCC CATGGGGCCA TGTTGGCCAC ATGCCCACGC
     AACGCAAGGC
 401 AGTAAGGCTG GCAGCCTGGC ACGCATTGAC GCATGTGGAC
     ACACACAGCC
```

451 GCCGCCTGTT CGTGTTTCTG TGCCGTTGTG CGAGACTGTG
ACTGCGAGTG
501 GCGGAGTCGG CGAACGGCGA GGCGTCTCCG GAGTCTGGAC
TGCGGCTGTG
551 GACAGCGACG CTGTGACGGC GACTCGGCGA AGCCCCAAGC
TACCAAGCCC
601 CCAAGTCCCC ATCCATCTCT GCTTCTCTGG TCATCTCCTT
CCCCTGGTCG
651 ATCTGCAGGC GCCAGACCG

Region 2 (SEQ ID NO: 29) Undescribed maize genomic sequence (complement)

670 G CCGAAGCATC ACGAAACGCA CTAAGACCTC
701 GAAGGAGTCA AACCACTCCT CCGAGGCCTC GGGGGCTACA
CCCGGCGGGT
751 GCGCTCGCGC GCACCCACCG GAACAAAATG TAACCGAGAA
AGGTCGGTCC
801 CCTTGCAAAA AAAGTGCGAC AAAAGCCTCC AAGCGAGTAT
TAACACTCAC
851 TTTGAGGCTC GGGGGCTAC

Region 3 (SEQ ID NO: 30) Fragment of maize Huck-1 retrotransposon

870 T GTCGGGGACC ATAATTAGGG GTACCCCCAA
901 GACTCCTAAT CTCAGCTGGT AACCCCCATC AGCACAAAGC
TGCAAAGGCC
951 TGATGGGTGC GATTAAGTCA AGGCTCGGTC CACTCAAGGG
ACACGATCTC
1001 GCCTCGCCCG AGCCCAGCCT CGGGCAAGGG CGGCCGACCC
CGAGGATTCA
1051 CGTCTCGCCC GAGGGCCCCC TCAAGCGACG GGCACACCTT
CGGCTCGCCC
1101 GAGGCCCATT CTTCGCCGAG AAGCAACCTT GGCCAGATCG
CCACACCGAC
1151 CGACCGTATC GCAGGAGCAT TTAATGCGAG GATCGCCTGA
CACCTTATCC
1201 TGACGCGCGC TCTTCAGTCG ACAGAGCCGA AGTGACCGCA
ATCACTTCGC
1251 CGCTCCACTG ACCGACCTGA CAAGAAGACA GCGCCGCCTG
CGTCGCTCCG
1301 ACTGCTGTGC CACTCGACAG AGTGAGGCTG ACAGCAGCCA
AGTCCGGCCT
1351 CGGGCGCCAT AGGAAGCTCC GCCTCGCCCG ACCCTAGGGC
TCGGACTCGG
1401 CCTCGGCTCC GGAAGACGAC GAACTACGCT TCGCCCGACC
CCAGGGCTTG
1451 GACTCAGCCT CGGCTCCGGA AGACGACGAA TTCCGCCTCG
CCCGACCCCA
1501 GGGCTCGGAC TCGGCCTCGG CTCCAGAAGA CGACGAACTC
CGCCTCGCCC

1551 GACCCCAGGG CTCGGACTCA GCCTCGGCTC CGGAAGACGA
CGAACTCCGC
1601 CTCGCCCGAC CCCAGGGCTC GGACTCAGCC TCGGCCTCAG
ACGATGGTCT
1651 CCGCCTCGCC CGACCCGGGG CTCGGACTCG A

Region 4 (SEQ ID NO: 31) Fragment of *cry*1F gene

1682 CCTTTCTAT CGGACCTTGT
1701 CAGATCCTGT CTTCGTCCGA GGAGGCTTTG GCAATCCTCA
CTATGTACTC
1751 GGTCTTAGGG GAGTGGCCTT TCAACAAACT GGTACGAATC
ACACCCGCAC
1801 ATTCAGGAAC TCCGGGACCA TTGACTCTCT AGATGAGATA
CCACCTCAAG
1851 ACAACAGCGG CGCACCTTGG AATGACTACT CCCATGTGCT
GAATCATGTT
1901 ACCTTTGTGC GCTGGCCAGG TGAGATCTCA GGTTCCGACT
CATGGAGAGC
1951 ACCAATGTTC TCTTGGACGC ATCGTAGCGC TACCCCCACA
AACACCATTG
2001 ATCCAGAGAG AATCAC

Region 5 (SEQ ID NO: 32) Fragment of maize chloroplast *rpo*C2 gene

2017 TCAT TCTTCAAGAA CTGCATATCT TGCCGAGATC
2051 CTCATCCCTA AAGGTACTTG ACAATAGTAT TATTGGAGTC
GATACACAAC
2101 TCACAAAAAA TACAAGAAGT CGACTAGGTG GATTGGTCCG
AGTGAAGAGA
2151 AAAAAAAGCC ATACAGAACT CAAAATCTTT TCCGGAGATA
TTCATTTTCC
2201 TGAAGAGGCG GATAAGATAT TAGGTGGCAG TTTGATACCA
CCAGAAAGAG
2251 AAAAAAAAGA TTCTAAGGAA TCAAAAAAAA GGAAAAATTG
GGTTTATGTT
2301 CAACGGAAAA AATTTCTCAA AAGCAAGGAA AAGTATT

Region 6 (SEQ ID NO: 33) Fragment of maize chloroplast or *ubi*ZM1(2) promoter

2338 GTG GCTATTTATC
2351 TATC

Nucleotides 2355-2358 (CGT) connect Region 6 to Region 7a.

Region 7a (SEQ ID NO: 34) Fragment of *pat* gene

2358 GCA GCTGATATGG CCGCGGTTTG TGATATCGTT AACCATTACA
2401 TTGAGACGTC TACAGTGAAC TTTAGGACAG AGCCACAAAC
ACCACAAGAG

2451 TGGATTGATG ATCTAGAGAG GTTGCAAGAT AGATACCCTT
GGTTGGTTGC
2501 TGAGGTTGAG GGTGTTGTGG CTGGTATTGC TTACGCTGGG
CCCTGGAAGG
2551 CTAGGAAC

Region 7b (SEQ ID NO: 35) Fragment of *pat* gene (complement)

2559 CC TCAACCTCAG CAACCAACCA ATGGTATCTA TCTTGCAACC
2601 TCTCTAGATC ATCAATCCAC TCTTGTGGTG TTTGTGGCTC
TGTCCTAAAG
2651 TTCACTGTAG ACGTCTCAAT GTAATGGTTA ACGATATCAC AAACCG

Region 7c (SEQ ID NO: 36) Fragment of *cry*1F gene (complement)

2697 AGAG
2701 AAGAGGGATC T

Region 8 (SEQ ID NO: 37) Fragment of Polylinker

2712 CGAAGCTTC GGCCGGGGCC CATCGATATC CGCGGGCATG
2751 CCTGCAGTGC AGCGTGACCC GGTCGTGCCC CTCTCTAGAG
ATAATGAGCA
2801 TTGCATGTCT AAGTTATAAA AAATTACCA

Region 9 (SEQ ID NO: 25) Full-length insert of PHI8999A

Example 5. Description of the flanking sequence 5' to the insert in maize event TC1507

In order to more fully describe the event TC1507 5' flanking sequence, homology searching was done against the GenBank public databases (release 122, 2/01) using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, pairwise alignments were performed using AlignX (InforMax Inc., Bethesda, MD) to look for homology between the maize event TC1507 flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The TC1507 5' flanking sequence is numbered with base 1 being the furthest 5' to the insert and base 2830 at the starting point of the full-length PHI8999A transgenic insert (see FIG. 1). The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed.

In most cases, similarity searching with the event TC1507 5' flanking sequence resulted in a match to one unique sequence based on a very high percent identity value. Those sequences are identified in Table 1. In addition, there are two regions in the

TC1507 5' DNA flanking sequence with high similarity to more than one known sequence. In regions 870-1681 and 2338-2354, the percent identity scores with both sequence fragments are sufficiently high that a single match (homolog) cannot be determined. The two possible homologs for each of these regions are indicated in Table 1.

Highly similar sequences were identified for all but the first 669 base pairs of sequence. Generally, the results of similarity searching indicate high homology with maize genomic sequences 5' to base 1681. The region from base 1682 to the start of the PHI8999A insert at position 2830 contains some fragments associated with the transformation event.

Table 1. Sequence summary for event TC1507 insert

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
1	1-669	669	N/A[1]	N/A	N/A	No significant homology detected
2	670-869	200	90.5	AF123535	52432-52632 (complement)	Undescribed maize genomic sequence
Region	**Location in SEQ ID NO:24**	**Size bp**	**% Identity**	**Homolog**	**Location in homologous sequence**	**Description**
3	870-1681	812	89.4	AF050439	1-801	Fragment of maize Huck-1 retrotransposon 5' LTR[2]
			86.6	AF050438	1-797	Fragment of maize Huck-1 retro-transposon 3' LTR
4	1682-2016	335	100.0	PHI8999A	3149-3483	Fragment of *cry*1F gene
5	2017-2337	321	100.0	X86563	29429-29749	Fragment of maize chloroplast *rpoC2* gene (RNA polymerase beta-2 subunit)
6	2338-2354	17	100.0	X86563	97643-97659	Fragment of maize chloroplast *trnI* gene (tRNA-Ile)

			82.4	PHI8999A	182-197	Fragment of maize *ubi*ZM1(2) promoter
7a	2358-2558	201	100.0	PHI8999A	5320-5475	Fragment of *pat* gene
7b	2559-2696	138	99	PHI8999A	5336-5518 (complement)	Fragment of *pat* gene
7c	2697-2711	15	100.0	PHI8999A	2544-2558 (complement)	Fragment of *cry*1F gene
8	2712-2829	118	100.0	PHI8999A	36-153	Fragment of polylinker region (bases 36-80) and *ubi*ZM1(2) promoter (bases 81-153)
9	2830-9015	6186	100.0	PHI8999A	11 – 6196	Full-length insert of PHI8999A
10	9016-9565	550	100.0	PHI8999A	3906-4456 (complement)	Inverted ORF25 terminator
11	9566-9693	128	100.0	NC_001666	121851-121978 (complement) & 100759-100886	Fragment of maize chloroplast *rps12* rRNA (23S ribosomal RNA)
12	9696-10087	392	99	NC_001666	17091-17483 (complement)	Fragment of maize chloroplast genome
13	10088-10275	188	99	PHI8999A	5333-5520 (complement)	Fragment of *pat* gene
14	10278-10358	81	100	NC_001666	137122-137202 (complement)	Fragment of maize chloroplast "ORF241" – hypothetical protein gene
15	10359-10612	254	N/A[1]	N/A	N/A	No significant homology detected
16	10613-11361	749	N/A[1]	N/A	N/A	No description available

[1]_ N/A; not applicable
[2]_ LTR; long terminal repeat

5

Example 6: Confirmation of the presence of Regions 1, 2, and 3 in an unmodified control corn line

PCR analysis was used to determine if Regions 1, 2, and 3 (Table 1) in the 5' flanking region of Event TC1507 are present in an unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Nine different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified control corn line Hi-II (see Armstrong (1994) *The Maize Handbook,* ed. Freeling and Walbot, Springer-Verlag, New York, pp. 663-671, for information on Hi-II) as outlined in Table 2 using the primer sequences shown in Table 3. Two reactions were designed to amplify DNA within Region 1 of the 5' flanking region from bp 25 to 324 (Reaction A - 300 bp amplicon); and from bp 25 to 480 (Reaction B - 456 bp amplicon). The expected amplicons were present in both the Hi-II unmodified corn line and in maize event TC1507. One PCR primer pair, Reaction C, spanned Region 2 to Region 3 of the 5' flanking region from bp 759 to 1182 (424 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reaction D, spanned Region 1 to Region 3 of the 5' flanking region from bp 415 to 1182 (768 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reactions E and F were designed as specific primer pairs for the *pat* gene region of the full-length insert of PHI8999A in TC1507 and thus an amplicon in the unmodified Hi-II corn line is not expected. The results indicate that both Reactions E and F are specific for a maize line transformed with a *pat* gene region and produce the expected amplicon, whereas no amplicon was produced in the unmodified Hi-II corn line. Reaction G was also designed as a primer pair that would produce an amplicon of 366 bp in the maize event TC1507 and no amplicon in the unmodified Hi-II corn line.

Reactions H and I were designed as specific primer pairs for TC1507 that would span the end of the transgenic insert into the 5' flanking region. In both Reactions H and I, the reverse primer was located in the ubiquitin promoter region of the full-length PHI8999A insert (Region 9 in Table 1) and the forward primer was located in Region 5, the *rpoC2* gene fragment (see Table 1). Reaction H and Reaction I both produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. These results indicate that both Reactions H and I are specific for the TC1507 event.

The PCR results show that the undescribed sequence (Region 1) is present in the unmodified corn line Hi-II and that Regions 1, 2 and 3, are contiguous in the unmodified

corn line Hi-II. The DNA sequences amplified in Reactions A, B, C, and D are not unique to the 5' flanking region of maize event TC1507 but are also present in the unmodified corn line Hi-II.

Table 2. PCR reactions for sequence 5' to the PHI8999A insert in maize event TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
A	25 – 324 bp in TC1507 flanking sequence	300	Region 1	Yes	Yes
B	25 – 480 bp in TC1507 flanking sequence	456	Region 1	Yes	Yes

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
	bp in TC1507 flanking sequence		3		
D	415 – 1182 bp in TC1507 5' flanking sequence	768	Region 1 to Region3	Yes	Yes
E Not Unique to TC1507	4750 – 5794 bp in PHI8999A	1045	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes
F Not Unique to TC1507	4827 - 5308 bp in PHI8999A	482	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
Detects cry1F fragment in 5' flanking region	*cry*1F sequence in 5' flanking sequence and in full-length insert of PHI8999A	366	Spans 335 bp *cry*1F sequence in 5' flanking sequence and same sequence in the full-length insert	No	Yes
H Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3069 bp in Region 9 (full-length insert of PHI8999A)	912	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes
I Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3001 bp in Region 9 (full-length insert of PHI8999A)	844	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes

Table 3. PCR primers for sequence 5' to the PHI8999A insert in TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
A	300	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:11	TTGATTGGCAGGTCCGTGGGTC
B	456	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:12	CACAACGGCACAGAAACACGAA
C	424	SEQ ID NO:13	GCGCACCCACCGGAACAAAATG
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
D	768	SEQ ID NO:15	CCTGGCACGCATTGACGCATGT
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
E	1045	SEQ ID NO:6	TAGAGGACCTAACAGAACTCGCCGT
		SEQ ID NO:7	GAGCTGGCAACTCAAAATCCCTTT
Reaction	**Amplicon Size (bp)**	**Primer Pair**	**Primer Sequences 5' to 3'**
F	482	SEQ ID NO:8	AAAATCTTCGTCAACATGGTGGAGC

		SEQ ID NO:9	TAATCTCAACTGGTCTCCTCTCCGG
G	366	SEQ ID NO:19	GGCTCGGACTCGACCTTTCTAT
		SEQ ID NO:20	GCAGTTCTTGAAGAATGAGTGA
H	912	SEQ ID NO:1	GTAGTACTATAGATTATATTATTCGT AGAG
		SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
I	844	SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
		SEQ ID NO:23	CTTCAAACAAGTGTGACAAA

Example 7. Flanking sequence 3' to inserted transgenic DNA in maize event TC1507

Two separate PCR approaches were used to extend the length of the sequence information 3' to the full-length PHI8999A insert in maize event TC1507. In the first approach PCR primer pairs were designed to amplify a product that spanned the junction between the full-length insert and the inverted ORF25 terminator, see FIG. 1 for a depiction of the inverted ORF25 terminator. A forward primer was located at the end of the full-length PHI8999A insert and a series of reverse primers were located at 100 bp intervals in the inverted sequence. In this manner the length of the inverted fragment present in the maize event TC1507 could be determined within a 100 bp region based on the successful PCR reactions. This method indicated the inverted fragment contained the majority of the ORF25 terminator but no Cry1F sequence. PCR fragments were isolated and sequenced from this region.

In the second approach PCR primers were designed to walk out into the flanking DNA sequence from the inverted ORF25 terminator region as determined in the PCR experiment described above. Genomic DNA isolated from two to three individual plants of event TC1507 and an unmodified control corn line was digested with various restriction enzymes and then ligated to adaptors specific for the restriction enzyme used for digestion (Universal Genome Walker™ Kit, Clontech Laboratories, Inc. and Devon *et al.* (1995) *Nucleic Acids Res.* 23:1644-1645). Primary PCR was carried out using an ORF25 terminator specific primer and a primer homologous to the adaptor sequence ligated onto the digested DNA. In order to increase the specificity of the reaction a nested secondary PCR was performed again with another ORF25 terminator specific primer and a secondary primer homologous to the adaptor sequence with the secondary primers being internal to the respective primers used in the primary PCR. Products produced by the nested PCR were analyzed by agarose gel electrophoresis and fragments unique to TC1507 DNA

samples were isolated and sequenced. Fragments were amplified from both the ORF25 terminator contained within the full-length insert and from the targeted (inverted) ORF25 terminator on the 3' end of the full-length PHI8999A insert. Fragments from the full-length insert were of a predicted size based on the knowledge of the restriction enzyme sites located in the full-length insert. Fragments produced from the 3' inverted ORF25 terminator appeared as fragments of unexpected size. Sequence analysis of amplified fragments from the 3' inverted ORF25 terminator resulted in flanking DNA sequence of 1043 bp. Resultant sequence from the above series of genome walking experiments was used to design additional primers to walk further out from the insert into the bordering maize genome with a final 3' flanking sequence, of 2346 bp.

In order to describe the TC1507 3' flanking sequence, homology searching was done against the GenBank public databases using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, alignments were performed using SeqMan 4.05™, Martinez and Needleman-Wunsch alignment algorithms (DNASTAR Inc.) to look for homology between the TC1507 3' flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed. The results of similarity searching for the 3' flanking sequence indicate high homology with three regions of maize chloroplast DNA, a 188 bp fragment of the *pat* gene, and 254 bp of DNA (Region 15, Table 1) with no significant homology. An additional 749 bp (Region 16) beyond Region 15 (see Table 1) was also sequenced. No similarity searching results are available for Region 16.

PCR analysis on control and TC1507 genomic DNA determined that the 254 bp sequence (Region 15, fragment of maize chloroplast "ORF241") is present in the maize genome. The DNA sequence of Region 15 in the 3' flanking region is not unique to the 3' flanking region of maize event TC1507 but is also present in the unmodified control corn line. The TC1507 3' flanking sequence is presented in Example 8 and diagrammed in FIG. 1.

Example 8. Sequence of the region 3' to the end of the full-length insert DNA in maize event TC1507. A description of each region is in Table 1.

Region 10 (SEQ ID NO: 38) Fragment of ORF25 Terminator (complement)

9016 CTCAC TCCGCTTGAT CTTGGCAAAG ATATTTGACG
9051 CATTTATTAG TATGTGTTAA TTTTCATTTG CAGTGCAGTA
TTTTCTATTC
9101 GATCTTTATG TAATTCGTTA CAATTAATAA ATATTCAAAT
CAGATTATTG
9151 ACTGTCATTT GTATCAAATC GTGTTTAATG GATATTTTTA
TTATAATATT
9201 GATGATATCT CAATCAAAAC GTAGATAATA ATAATATTTA
TTTAATATTT
9251 TTGCGTCGCA CAGTGAAAAT CTATATGAGA TTACAAAATA
CCGACAACAT
9301 TATTTAAGAA ACATAGACAT TAACCCTGAG ACTGTTGGAC
ATCAACGGGT
9351 AGATTCCTTC ATGCATAGCA CCTCATTCTT GGGGACAAAA
GCACGGTTTG
9401 GCCGTTCCAT TGCTGCACGA ACGAGCTTTG CTATATCCTC
GGGTTGGATC
9451 ATCTCATCAG GTCCAATCAA ATTTGTCCAA GAACTCATGT
TAGTCGCAAC
9501 GAAACCGGGG CATATGTCGG GTATCTCGAG CTCGCGAAAG
CTTGGCTGCA
9551 GGTCGACGGA TCCTT

Region 11 (SEQ ID NO: 39) Fragment of maize chloroplast *rps12* rRNA gene (complement)

9566 CAACA AAAGGGTACC TGTACCCGAA ACCGACACAG
9601 GTGGGTAGGT AGAGAATACC TAGGGGCGCG AGACAACTCT
CTCTAAGGAA
9651 CTCGGCAAAA TAGCCCCGTA ACTTCGGGAG AAGGGGTGCC CCC

Nucleotides 9694-9695 (CG) connect Region 11 to Region 12.

Region 12 (SEQ ID NO: 40) Fragment of maize chloroplast genome

9696 CTAAC
9701 AATAAACGAA TACGGTTTAT GTATGGATTC CGGTAAAATA
CCGGTACTCG
9751 ATTTCATAAG AGTCGAATAG GAAGTTAAGA TGAGGGTGGT
ATCATCATAA
9801 AAATGGAGTA GTATCCTAAA TTATACTAAT CCACGTATGA
TATGTATGCC
9851 TTTCCTTATC AACCGGAAGT AGTGCAAAAA AAATTCTATA
CTGCACTGCT
9901 CTCTTTTTAC TGAGAAATGC AAAAAAATAA AAGTGAAGTA
AGGGTGCCCC

9951 ATAGATATTT GATCTTGCCT CCTGTCCCCC CCCCCCTTTT
TTCATCAAAA
10001 ATTTCCATGA AAAAAGAAAA GATGAATTTG TCCATTCATT
GAACCCTAGT
10051 TCGGGACTGA CGGGGCTCGA ACCCGCAGCT TCCGCCT

Region 13 (SEQ ID NO: 41) **Fragment of *pat* gene (complement)**

10088 GTT CCTAGCCTTC
10101 CAGGGCCCAG CGTAAGCAAT ACCAGCCACA GCACCCTCAA
CCTCAGCAAC
10151 CAACCAAGGG TATCTATCTT GCAACCTCTC TAGATCATCA
ATCCACTCTT
10201 GTGGTGTTTG TGGCTCTGTC CTAAAGTTCA CTGTAGACGT
CTCAATGTAA
10251 TGGTTAACGA TATCACAAAC CGCGG

Nucleotides 10276-10277 (AA) connect Region 13 to Region 14.

Region 14 (SEQ ID NO: 42) Fragment of maize chloroplast ORF241 (complement)

10278 CAC AAGAACGAAA GCACCTTTTC
10301 ATTCTTTCAT ATACTAGGGG TTTTTACTTG GAAAAGACAA
TGTTCCATAC
10351 TAAAGGAT

Region 15 (SEQ ID NO: 43) **Maize genomic (no significant homology)**

10359 AG CTGCAGAAGC CGCCACCGTC TTGAGGACCT TCCGGGGAGC
10401 CAGACCGGTC GAACCGTGCC TCCACTTGCT AAGGAGAAAG
GGAAAATCAG
10451 GGCCAGGACA TACGAAGGAG GAGCCAGAAC GAAGATATCC
TAAGATACTT
10501 ACTCGCTCCG GGCCATGATC AATCATGCCT GTGGGGAGGT
CTCTCGCACC
10551 TCGATCCATG AAGGTACCAC CGAGGTCTGC CCCGCCGCCG
GCTTCGGTAC
10601 CGTCCTCGCC TT

Region 16 (SEQ ID NO: 44) Maize genomic

10613 GGGCGCCC GAGGCACCCG GGGGATGGAC TGCCCAGGCG
10651 CAGCCACGAC GACCCAAGGA TCACCCTCCT GCGCAGTCGG
CACGAGCAAT
10701 AGTTCTCGGG GAACAGGCAG CTTGGCCTGA CTCCCCGGGG
TCACCTCAAC
10751 TACCTCGGCC GAGGGGTCAA GTACCCCCTC AGTCCGCCCC
CGCTCTTCGG
10801 ACCGGGACCC CGACGTCCCG GCCCCGGATA CCGACGGCAC
CAGCCCGCTC

10851 GGGGGCTGGC TTGACGACCC CTGGCCCAGC CTCAGATCTG
GGCTGAGGCC
10901 GAGGCAGGCG GCCATGTCGT CGTCTTCATC ATCGTCTTCA
TCATCGTCGT
10951 CGTCATCAGG CGTCTCCGGC GACGGCTCCC TTGGGAGCCC
CTCCCTCTCC
11001 TGCCGACGAC GAAGCCTTTC CAAGGCATCC CGAGCCCACG
TCCGCTCGTG
11051 GGCCCGAGCC TTCTTTGCGT CCTTCTTCTC CTTCCTCTTC
TCCGCGGTGA
11101 CCCTCCGCGC AGCTCGGTCC ACCGCATCCT CCGGGACTGG
TGGCAGGGAA
11151 GGCTTGTGAT GCCCTACCTC CTGGAGACAG ACGAAAAGTC
TCAGCTATGA
11201 GAACCGAGGG CAATCTGACG CAAGAAGGAA GAAGGAGCGG
ATACTCACCA
11251 GAGACACGCA CCCGCGATCG GGACGCATTA AGGGCTGGGA
AAAAGTGCCG
11301 GCCTCTAATT TCGCTACCGT GCCGTCCACC CACCTGTGGA
GGTCATCGAT
11351 GGGAAGGGGA A

Example 9. Confirmation of the presence of Region 15 in the unmodified control corn line

PCR analysis was used to determine if the undescribed region of sequence on the end of the 3' flanking sequence (Region 15 in Table 1) is present in the unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Successful PCR amplification of Region 15 in both maize line TC1507 and the unmodified Hi-II control corn line revealed that Region 15 was indeed present in corn genomic DNA. Five different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified Hi-II control corn line as outlined in Table 7 below using the primer sequences shown in Table 8. Three reactions were designed to amplify DNA within Region 15 of the 3' flanking region; Reaction L – producing a 175 bp amplicon, Reaction M – producing a 134 bp amplicon, and Reaction N – producing a 107 bp amplicon. The expected amplicons were present in both the unmodified control corn line and in maize line TC1507. Reactions J and K were designed as specific primer pairs for TC1507 that would span the end of the insert into the 3' flanking region. In Reaction J, the forward primer was located in the *pat* gene fragment on the 3' end of the full-length PHI8999A insert (Region 13 in Table 1) and the reverse primer was located in the undefined Region 15. In Reaction K the forward primer was

located in the chloroplast hypothetical protein gene on the 3' end of the full-length insert (Region 14 in Table 1) and the reverse primer was located in the undefined Region 15. Both Reaction J and Reaction K produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. The results indicate that both Reactions J and K are specific for the TC1507 event.

The PCR results indicate that the undescribed sequence (Region 15) of the 3' flanking sequence of TC1507 is also present in genomic DNA isolated from the unmodified Hi-II control corn line. The DNA sequences amplified in Reactions L, M, and N are not unique to the 3' flanking region of TC1507 but are also present in the unmodified control corn line.

Table 7. PCR reactions for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Region in TC1507 3' flanking sequence	Amplicon present in Control	Amplicon present in maize line TC1507
J	342	Region 13 (*pat* gene fragment) to Region 15	No	Yes
K	252	Region 14 (chloroplast gene) to Region 15	No	Yes
L	175	Region 15	Yes	Yes
M	134	Region 15	Yes	Yes
N	107	Region 15	Yes	Yes

Table 8. PCR primers for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
J	342	SEQ ID NO:3	TGTGGTGTTTGTGGCTCTGTCCTAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
K	252	SEQ ID NO:4	AGCACCTTTTCATTCTTTCATATAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
L	175	SEQ ID NO:16	AAGCCGCCACCGTCTTGAGGACCTT

		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
M	134	SEQ ID NO:17	GTCGAACCGTGCCTCCACTTGC TAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
N	107	SEQ ID NO:18	AGAAAGGGAAAATCAGGGCCA GGAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC

Example 10. PCR Primers

DNA event specific primer pairs were used to produce an amplicon diagnostic for TC1507. These event primer pairs include, but are not limited to, SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 2 and SEQ ID NO: 23; SEQ ID NO: 3 and SEQ ID NO: 5; and SEQ ID NO: 4 and SEQ ID NO: 5. In addition to these primer pairs, any primer pair derived from SEQ ID NO: 26 and SEQ ID NO: 27 that when used in a DNA amplification reaction produces a DNA amplicon diagnostic for TC1507 is disclosed. The amplification conditions for this analysis are illustrated in Table 9, however, any modification of these methods that use DNA primers or complements thereof to produce an amplicon DNA molecule diagnostic for TC1507 is within the ordinary skill of the art. The preferred amplification conditions for reactions utilizing the PCR primers identified in SEQ ID NOS: 1, 2, and 23 are illustrated in Table 10. In addition, control primer pairs, which include SEQ ID NOS: 10 and 11; SEQ ID NOS: 10 and 12; SEQ ID NOS: 13 and 14; SEQ ID NOS: 14 and 15; SEQ ID NOS: 5 and 16; SEQ ID NOS: 5 and 17; and SEQ ID NOS: 5 and 18; for amplification of an endogenous corn gene are included as internal standards for the reaction conditions. Also included are primer pairs that will produce an amplicon in transgenic events containing a *pat* gene (SEQ ID NOS: 6 and 7; SEQ ID NOS: 8 and 9), and a primer pair that will produce an amplicon in transgenic events containing a *cry*1F gene (SEQ ID NOS: 19 and 20).

The analysis of plant tissue DNA extracts to test for the presence of the TC1507 event should include a positive tissue DNA extract control (a DNA sample known to contain the transgenic sequences). A successful amplification of the positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences. A negative, or wild-type, DNA extract control in which the template DNA provided is either genomic DNA prepared from a non-transgenic plant, or is a non-TC1507 transgenic plant, should also be included. Additionally a negative control that

contains no template corn DNA extract will be a useful gauge of the reagents and conditions used in the PCR protocol.

Additional DNA primer molecules of sufficient length can be selected from SEQ ID NO: 26 and SEQ ID NO: 27 by those skilled in the art of DNA amplification methods, and conditions optimized for the production of an amplicon that may differ from the methods shown in Table 9 or Table 10 but result in an amplicon diagnostic for event TC1507. The amplicon wherein at least one DNA primer molecule of sufficient length derived from SEQ ID NO: 26 and SEQ ID NO: 27 that is diagnostic for event TC1507 is disclosed. The amplicon wherein at least one DNA primer of sufficient length derived from any of the genetic elements of PHI8999A that is diagnostic for event TC1507 is disclosed. The assay for the TC1507 amplicon can be performed by using a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler, or by methods and apparatus known to those skilled in the art.

Table 9. PCR Conditions:

Conditions:

Kit used:	Perkin-Elmer AmpliTAQ Gold kit
Volume	Component
5 µl	template (10 ng/µl)
4 µl	2 µl each primer (10 µM)
2 µl	10X PCR Gold Buffer
2 µl	25 mM $MgCl_2$
2 µl	50X dNTP's (10 mM)
0.1 µl	Amplitaq Gold Polymerase
4.9 µl	H_2O
20 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

9 min 92°C
30 cycles:
94°C 30 sec
60°C 30 sec
72°C 1 min

7 min 72°C
Hold 4°C

Table 10. PCR Conditions used with the Advantage®-GC 2 Polymerase Mix:

Conditions:

Kit used:	Advantage®-GC 2 Polymerase Mix
Volume	Component
5 µl	template (10 ng/µl)
5 µl	2.5 µl each primer (10 µM)
10 µl	5x GC2 Buffer
10 µl	GC melt (1.0 M final conc.)
1.5 µl	50X dNTP's (10 mM)
1.0 µl	Advantage GC2 Polymerase
17.5 µl	H_2O
50 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

5 min 94°C
35 cycles:
94°C 1 min
60°C 2 min
72°C 3 min
7 min 72°C
Hold 4°C

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles.

CLAIMS

1. An insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

2. The plant or seed of claim 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

~~CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF~~

FIELD OF THE INVENTION

The present invention relates to the field of plant molecular biology, specifically the invention relates to a DNA construct for conferring insect resistance to a plant. The invention more specifically relates to an insect resistant corn plant TC1507~~ and to assays for detecting the presence of corn plant TC1507 DNA in a sample and compositions thereof~~.

BACKGROUND OF THE INVENTION

This invention relates to the insect resistant corn (*Zea mays*) plant TC1507, also referred to as maize line TC1507 or maize event TC1507~~, and to the DNA plant expression construct of corn plant TC1507 and the detection of the transgene/flanking insertion region in corn plant TC1507 and progeny thereof~~.

Corn is an important crop and is a primary food source in many areas of the world. Damage caused by insect pests is a major factor in the loss of the world's corn crops, despite the use of protective measures such as chemical pesticides. In view of this, insect resistance has been genetically engineered into crops such as corn in order to control insect damage and to reduce the need for traditional chemical pesticides. One group of genes which have been utilized for the production of transgenic insect resistant crops are the delta-endotoxins from *Bacillus thuringiensis (B.t.)*. Delta-endotoxins have been successfully expressed in crop plants such as cotton, potatoes, rice, sunflower, as well as corn, and have proven to provide excellent control over insect pests. (Perlak, F.J *et al.* (1990) *Bio/Technology* 8, 939-943; Perlak, F.J. *et al.* (1993) *Plant Mol. Biol.* 22: 313-321; Fujimoto H. *et al.* (1993) *Bio/Technology* 11: 1151-1155; Tu *et al.* (2000) *Nature Biotechnology* 18:1101-1104; PCT publication number WO 01/13731; and Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, Fort Collins, CO).

The expression of foreign genes in plants is known to be influenced by their location in the plant genome, perhaps due to chromatin structure (e.g., heterochromatin) or the proximity of transcriptional regulatory elements (e.g., enhancers) close to the integration site (Weising *et al.*, *Ann. Rev. Genet* 22:421-477, 1988). At the same time the presence of the transgene at different locations in the genome will influence the overall

phenotype of the plant in different ways. For this reason, it is often necessary to screen a large number of events in order to identify an event characterized by optimal expression of an introduced gene of interest. For example, it has been observed in plants and in other organisms that there may be a wide variation in levels of expression of an introduced gene among events. There may also be differences in spatial or temporal patterns of expression, for example, differences in the relative expression of a transgene in various plant tissues, that may not correspond to the patterns expected from transcriptional regulatory elements present in the introduced gene construct. For this reason, it is common to produce hundreds to thousands of different events and screen those events for a single event that has desired transgene expression levels and patterns for commercial purposes. An event that has desired levels or patterns of transgene expression is useful for introgressing the transgene into other genetic backgrounds by sexual outcrossing using conventional breeding methods. Progeny of such crosses maintain the transgene expression characteristics of the original transformant. This strategy is used to ensure reliable gene expression in a number of varieties that are well adapted to local growing conditions.

It would be advantageous to be able to detect the presence of a particular event in order to determine whether progeny of a sexual cross contain a transgene of interest. In addition, a method for detecting a particular event would be helpful for complying with regulations requiring the pre-market approval and labeling of foods derived from recombinant crop plants, for example, or for use in environmental monitoring, monitoring traits in crops in the field, or monitoring products derived from a crop harvest, as well as for use in ensuring compliance of parties subject to regulatory or contractual terms.

It is possible to detect the presence of a transgene by any nucleic acid detection method known in the art including, but not limited to, the polymerase chain reaction (PCR) or DNA hybridization using nucleic acid probes. These detection methods generally focus on frequently used genetic elements, such as promoters, terminators, marker genes, etc., because for many DNA constructs, the coding region is interchangeable. As a result, such methods may not be useful for discriminating between different events, particularly those produced using the same DNA construct or very similar constructs unless the DNA sequence of the flanking DNA adjacent to the inserted heterologous DNA is known. For example, an event-specific PCR assay is described in U.S. Patent No. 6,395,485 for the detection of elite event GAT-ZM1. Accordingly, it would be desirable to have a simple and discriminative method for the identification of event TC1507.

SUMMARY OF THE INVENTION

This disclosure~~invention~~ relates preferably to methods for producing and selecting an insect resistant monocot crop plant. More specifically, a DNA construct is provided that when expressed in plant cells and plants confers resistance to insects. Accordingly ~~to one aspect of the invention,~~ a DNA construct, capable of introduction into and replication in a host cell, is provided that when expressed in plant cells and plants confers insect resistance to the plant cells and plants. The DNA construct is comprised of a DNA molecule named PHI8999A and it includes two transgene expression cassettes. The first expression cassette comprises a DNA molecule which includes the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) *Plant Mol. Biol.* 18:675-689 and Christensen and Quail (1996) *Transgenic Res.* 5:213-218) operably connected to a DNA molecule encoding a *B.t.* δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350). The second transgene expression cassette of the DNA construct comprises a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59) operably connected to a DNA molecule encoding a phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene* 70: 25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37: 49-59). Plants containing the DNA construct are also provided.

One aspect of the invention is an insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

Another aspect of the invention is the plant or seed of aspect 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

~~According to another aspect of the invention, compositions and methods are provided for identifying a novel corn plant designated TC1507, which methods are based on primers or probes which specifically recognize the 5' and/or 3' flanking sequence of TC1507. DNA molecules are provided that comprise primer sequences that when utilized in a PCR reaction will produce amplicons unique to the transgenic event TC1507. These molecules may be selected from the group consisting of:~~

~~5'-GTAGTACTATAGATTATATTATTCGTAGAG-3' (SEQ ID NO: 1);~~

~~5'-GCCATACAGAACTCAAAATCTTTTCCGGAG-3' (SEQ ID NO: 2);~~

~~5'- CTTCAAACAAGTGTGACAAA 3' (SEQ ID NO: 23);~~

~~5'-TGTGGTGTTTGTGGCTCTGTCCTAA-3' (SEQ ID NO: 3);~~

~~5'-AGCACCTTTTCATTCTTTCATATAC-3' (SEQ ID NO: 4);~~

~~5'-GACCTCCCCA CAGGCATGAT TGATC-3' (SEQ ID NO: 5);~~

~~and complements thereof. The corn plant and seed comprising these molecules is an aspect of this invention. Further, kits utilizing these primer sequences for the identification of the TC1507 event are provided.~~

~~An additional aspect of the invention relates to the specific flanking sequences of TC1507 described herein, which can be used to develop specific identification methods for TC1507 in biological samples. More particularly, the invention relates to the 5' and/or 3' flanking regions of TC1507, SEQ ID NO:21 and SEQ ID NO:22, respectively, which can be used for the development of specific primers and probes. The invention further relates to identification methods for the presence of TC1507 in biological samples based on the use of such specific primers or probes.~~

~~According to another aspect of the invention, methods of detecting the presence of DNA corresponding to the corn event TC1507 in a sample are provided. Such methods comprise: (a) contacting the sample comprising DNA with a DNA primer set, that when used in a nucleic acid amplification reaction with genomic DNA extracted from corn event TC1507 produces an amplicon that is diagnostic for corn event TC1507; (b) performing a~~

nucleic acid amplification reaction, thereby producing the amplicon; and (c) detecting the amplicon.

DNA molecules that comprise the novel transgene/flanking insertion region, SEQ ID NO: 26 and SEQ ID NO: 27 and are homologous or complementary to SEQ ID NO: 26 and SEQ ID NO: 27 are an aspect of this invention.

DNA sequences that comprise the novel transgene/flanking insertion region, SEQ ID NO:26 are an aspect of this invention. DNA sequences that comprise a sufficient length of polynucleotides of transgene insert sequence and a sufficient length of polynucleotides of maize genomic and/or flanking sequence from maize plant TC1507 of SEQ ID NO:26 that are useful as primer sequences for the production of an amplicon product diagnostic for maize plant TC1507 are included.

In addition, DNA sequences that comprise the novel transgene/flanking insertion region, SEQ ID NO:27 are provided. DNA sequences that comprise a sufficient length of polynucleotides of transgene insert sequence and a sufficient length of polynucleotides of maize genomic and/or flanking sequence from maize plant TC1507 of SEQ ID NO:27 that are useful as primer sequences for the production of an amplicon product diagnostic for maize plant TC1507 are included.

According to another aspect of the invention, the DNA sequences that comprise at least 11 or more nucleotides of the transgene portion of the DNA sequence of SEQ ID NO:26 or complements thereof, and a similar length of 5' flanking maize DNA sequence of SEQ ID NO:26 or complements thereof are useful as DNA primers in DNA amplification methods. The amplicons produced using these primers are diagnostic for maize event TC1507. Therefore, the invention also includes the amplicons produced by DNA primers homologous or complementary to SEQ ID NO:26.

According to another aspect of the invention, the DNA sequences that comprise at least 11 or more nucleotides of the transgene portion of the DNA sequence of SEQ ID NO:27 or complements thereof, and a similar length of 3' flanking maize DNA sequence of SEQ ID NO:27 or complements thereof are useful as DNA primers in DNA amplification methods. The amplicons produced using these primers are diagnostic for maize event TC1507. Therefore, the invention also includes the amplicons produced by DNA primers homologous or complementary to SEQ ID NO:27.

More specifically, a pair of DNA molecules comprising a DNA primer set, wherein the DNA molecules are identified as SEQ ID NO: 1 or complements thereof and SEQ ID

NO: 2 or complements thereof; SEQ ID NO: 2 or complements thereof and SEQ ID NO: 23 or complements thereof; SEQ ID NO: 3 or complements thereof and SEQ ID NO: 5 or complements thereof; SEQ ID NO: 4 or complements thereof and SEQ ID NO: 5 or complements thereof are aspects of the invention.

Further aspects of the invention include the amplicon comprising the DNA molecules of SEQ ID NO: 1 and SEQ ID NO: 2; the amplicon comprising the DNA molecules of SEQ ID NO: 2 and SEQ ID NO: 23; the amplicon comprising the DNA molecules of SEQ ID NO: 3 and SEQ ID NO: 5; and the amplicon comprising the DNA molecules of SEQ ID NO: 4 and SEQ ID NO: 5.

According to another aspect of the invention, methods of detecting the presence of a DNA molecule corresponding to the TC1507 event in a sample, such methods comprising: (a) contacting the sample comprising DNA extracted from a corn plant with a DNA probe, molecule that hybridizes under stringent hybridization conditions with DNA extracted from corn event TC1507 and does not hybridize under the stringent hybridization conditions with a control corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA. More specifically, a method for detecting the presence of a DNA molecule corresponding to the TC1507 event in a sample, such methods, consisting of (a) contacting the sample comprising DNA extracted from a corn plant with a DNA probe molecule that consists of sequences that are unique to the event, e.g. junction sequences, wherein said DNA probe molecule hybridizes under stringent hybridization conditions with DNA extracted from corn event TC1507 and does not hybridize under the stringent hybridization conditions with a control corn plant DNA; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA.

In addition, a kit and methods for identifying event TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24 are provided.

DNA molecules are provided that comprise at least one junction sequence of TC1507 selected from the group consisting of SEQ ID NO:45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof; wherein a junction sequence spans the junction between heterologous DNA inserted into the genome and the DNA from the corn cell flanking the insertion site, i.e. flanking DNA, and is diagnostic for the TC1507 event.

According to another aspect of the invention, methods of producing an insect resistant corn plant that comprise the steps of: (a) sexually crossing a first parental corn

line comprising the expression cassettes of the present invention, which confers resistance to insects, and a second parental corn line that lacks insect resistance, thereby producing a plurality of progeny plants; and (b) selecting a progeny plant that is insect resistant. Such methods may optionally comprise the further step of back-crossing the progeny plant to the second parental corn line to producing a true-breeding corn plant that is insect resistant.

The present invention provides a method of producing a corn plant that is resistant to insects comprising transforming a corn cell with the DNA construct PHI8999A (SEQ ID NO:25), growing the transformed corn cell into a corn plant, selecting the corn plant that shows resistance to insects, and further growing the corn plant into a fertile corn plant. The fertile corn plant can be self pollinated or crossed with compatible corn varieties to produce insect resistant progeny.

The invention further relates to a DNA detection kit for identifying maize event TC1507 in biological samples. Preferably the kit of the invention comprises a first primer which specifically recognizes the 5' or 3' flanking region of TC1507, and a second primer which specifically recognizes a sequence within the foreign DNA of TC1507, or within the flanking DNA, for use in a PCR identification protocol. The invention also relates to a kit for identifying event TC1507 in biological samples, which kit comprises a specific probe having a sequence which corresponds or is complementary to, a sequence having between 80% and 100% sequence identity with a specific region of event TC1507. Preferably the sequence of the probe corresponds to a specific region comprising part of the 5' or 3' flanking region of event TC1507.

The methods and kits encompassed by the present invention can be used for different purposes such as, but not limited to the following: to identify event TC1507 in plants, plant material or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material; additionally or alternatively, the methods and kits of the present invention can be used to identify transgenic plant material for purposes of segregation between transgenic and non-transgenic material; additionally or alternatively, the methods and kits of the present invention can be used to determine the quality of plant material comprising maize event TC1507. The kits may also contain the reagents and materials necessary for the performance of the detection method.

This invention further relates to the TC1507 corn plant or its parts, including, but not limited to, pollen, ovules, vegetative cells, the nuclei of pollen cells, and the nuclei of egg cells of the corn plant TC1507 and the progeny derived thereof. The corn plant and

~~seed TC1507 from which the DNA primer molecules of the present invention provide a specific amplicon product is an aspect of the invention.~~

The foregoing and other aspects of the invention will become more apparent from the following detailed description and accompanying drawing.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Linear map showing the transgenic insert PHI8999A, as well as the sequences flanking the transgenic insert.

DETAILED DESCRIPTION

The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al.*, *Glossary of Genetics: Classical and Molecular, 5th edition,* Springer-Verlag; New York, 1991; and Lewin, *Genes V,* Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at 37 CFR 1.822 is used.

As used herein, the term "comprising" means "including but not limited to".

As used herein, the term "corn" means *Zea mays* or maize and includes all plant varieties that can be bred with corn, including wild maize species.

As used herein, the term "TC1507 specific" refers to a nucleotide sequence which is suitable for discriminatively identifying event TC1507 in plants, plant material, or in products such as, but not limited to, food or feed products (fresh or processed) comprising, or derived from plant material.

As used herein, the terms "insect resistant" and "impacting insect pests" refers to effecting changes in insect feeding, growth, and/or behavior at any stage of development, including but not limited to: killing the insect; retarding growth; preventing reproductive capability; and the like.

As used herein, the terms "pesticidal activity" and "insecticidal activity" are used synonymously to refer to activity of an organism or a substance (such as, for example, a protein) that can be measured by numerous parameters including, but not limited to, pest mortality, pest weight loss, pest attraction, pest repellency, and other behavioral and

physical changes of a pest after feeding on and/or exposure to the organism or substance for an appropriate length of time. For example "pesticidal proteins" are proteins that display pesticidal activity by themselves or in combination with other proteins.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. As used herein, the terms "encoding" or "encoded" when used in the context of a specified nucleic acid mean that the nucleic acid comprises the requisite information to guide translation of the nucleotide sequence into a specified protein. The information by which a protein is encoded is specified by the use of codons. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid or may lack such intervening non-translated sequences (e.g., as in cDNA).

"Gene" refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. "Native gene" refers to a gene as found in nature with its own regulatory sequences. "Chimeric gene" refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. "Endogenous gene" refers to a native gene in its natural location in the genome of an organism. "Foreign" refers to material not normally found in the location of interest. Thus "foreign DNA" may comprise both recombinant DNA as well as newly introduced, rearranged DNA of the plant. A "foreign" gene refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, or chimeric genes. A "transgene" is a gene that has been introduced into the genome by a transformation procedure. The site in the plant genome where a recombinant DNA has been inserted may be referred to as the "insertion site" or "target site".

As used herein, "insert DNA" refers to the heterologous DNA within the expression cassettes used to transform the plant material while "flanking DNA" can exist of either genomic DNA naturally present in an organism such as a plant, or foreign (heterologous) DNA introduced via the transformation process which is extraneous to the original insert DNA molecule, e.g. fragments associated with the transformation event. A "flanking

region" or "flanking sequence" as used herein refers to a sequence of at least 20 base pair, preferably at least 50 base pair, and up to 5000 base pair which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the original foreign insert DNA molecule. Transformation procedures leading to random integration of the foreign DNA will result in transformants containing different flanking regions characteristic and unique for each transformant. When recombinant DNA is introduced into a plant through traditional crossing, its flanking regions will generally not be changed. Transformants will also contain unique junctions between a piece of heterologous insert DNA and genomic DNA, or 2 pieces of genomic DNA, or 2 pieces of heterologous DNA. A "junction" is a point where 2 specific DNA fragments join. For example, a junction exists where insert DNA joins flanking DNA. A junction point also exists in a transformed organism where 2 DNA fragments join together in a manner that is modified from that found in the native organism. "Junction DNA" refers to DNA that comprises a junction point.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous nucleotide sequence can be from a species different from that from which the nucleotide sequence was derived, or, if from the same species, the promoter is not naturally found operably linked to the nucleotide sequence. A heterologous protein may originate from a foreign species, or, if from the same species, is substantially modified from its original form by deliberate human intervention.

"Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements are often referred to as enhancers. Accordingly, an

"enhancer" is a nucleotide sequence that can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. Promoters that cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

The "translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect numerous parameters including, processing of the primary transcript to mRNA, mRNA stability and/or translation efficiency. Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

The "3' non-coding sequences" refer to nucleotide sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3' end of the mRNA precursor. The use of different 3' non-coding sequences is exemplified by Ingelbrecht *et al.* (1989) *Plant Cell* 1:671-680.

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide.

A DNA construct is an assembly of DNA molecules linked together that provide one or more expression cassettes. The DNA construct may be a plasmid that is enabled for self replication in a bacterial cell and contains various endonuclease enzyme restriction sites that are useful for introducing DNA molecules that provide functional genetic

elements, i.e., promoters, introns, leaders, coding sequences, 3' termination regions, among others; or a DNA construct may be a linear assembly of DNA molecules, such as an expression cassette. The expression cassette contained within a DNA construct comprise the necessary genetic elements to provide transcription of a messenger RNA. The expression cassette can be designed to express in prokaryote cells or eukaryotic cells~~.~~ ~~Expression cassettes of the present invention are designed to express~~ most preferably in plant cells.

The DNA molecules ~~of the invention~~ are provided in expression cassettes for expression in an organism of interest. The cassette will include 5' and 3' regulatory sequences operably linked to a coding sequence ~~of the invention~~. "Operably linked" means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. Operably linked is intended to indicate a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes or multiple DNA constructs.

The expression cassette will include in the 5' to 3' direction of transcription: a transcriptional and translational initiation region, a coding region, and a transcriptional and translational termination region functional in the organism serving as a host. The transcriptional initiation region (*i.e.*, the promoter) may be native or analogous, or foreign or heterologous to the host organism. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation.

It is to be understood that as used herein the term "transgenic" includes any cell, cell line, callus, tissue, plant part, or plant the genotype of which has been altered by the presence of a heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral

infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

A transgenic "event" is produced by transformation of plant cells with a heterologous DNA construct(s), including a nucleic acid expression cassette that comprises a transgene of interest, the regeneration of a population of plants resulting from the insertion of the transgene into the genome of the plant, and selection of a particular plant characterized by insertion into a particular genome location. An event is characterized phenotypically by the expression of the transgene. At the genetic level, an event is part of the genetic makeup of a plant. The term "event" also refers to progeny produced by a sexual outcross between the transformant and another variety that include the heterologous DNA. Even after repeated back-crossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed parent is present in the progeny of the cross at the same chromosomal location. The term "event" also refers to DNA from the original transformant comprising the inserted DNA and flanking sequence immediately adjacent to the inserted DNA that would be expected to be transferred to a progeny that receives inserted DNA including the transgene of interest as the result of a sexual cross of one parental line that includes the inserted DNA (e.g., the original transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA.

An insect resistant TC1507 corn plant can be bred by first sexually crossing a first parental corn plant consisting of a corn plant grown from the transgenic TC1507 corn plant and progeny thereof derived from transformation with the expression cassettes ~~of the present invention~~ that confers insect resistance, and a second parental corn plant that lacks insect resistance, thereby producing a plurality of first progeny plants; and then selecting a first progeny plant that is resistant to insects; and selfing the first progeny plant, thereby producing a plurality of second progeny plants; and then selecting from the second progeny plants an insect resistant plant. These steps can further include the back-crossing of the first insect resistant progeny plant or the second insect resistant progeny plant to the second parental corn plant or a third parental corn plant, thereby producing a corn plant that is resistant to insects.

As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, and progeny of same. Parts of transgenic plants understood to be within the scope of the invention comprise, for example, plant cells, protoplasts, tissues, callus, embryos as well as flowers, stems, fruits,

leaves, and roots originating in transgenic plants or their progeny previously transformed with a DNA molecule of the invention and therefore consisting at least in part of transgenic cells, are also an aspect of the present invention.

As used herein, the term "plant cell" includes, without limitation, seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants that can be used in the methods ~~of the invention~~ is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants.

"Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere *et al*. (1987) *Meth. Enzymol*. 143:277) and particle-accelerated or "gene gun" transformation technology (Klein *et al*. (1987) *Nature* (London) 327:70-73; U.S. Patent No. 4,945,050~~, incorporated herein by reference~~). Additional transformation methods are disclosed below.

Thus, isolated polynucleotides ~~of the present invention~~ can be incorporated into recombinant constructs, typically DNA constructs, which are capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of transgenic plants have been described in, e.g., Pouwels *et al*., (1985; Supp. 1987) *Cloning Vectors: A Laboratory Manual*, Weissbach and Weissbach (1989) *Methods for Plant Molecular Biology*, (Academic Press, New York); and Flevin *et al*., (1990) *Plant Molecular Biology Manual*, (Kluwer Academic Publishers). Typically, plant expression vectors include, for example, one or more cloned plant genes under the transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

It is also to be understood that two different transgenic plants can also be mated to produce offspring that contain two independently segregating added, exogenous genes. Selfing of appropriate progeny can produce plants that are homozygous for both added, exogenous genes. Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated, as is vegetative propagation. Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several references, e.g., Fehr, in *Breeding Methods for Cultivar Development*, Wilcos J. ed., American Society of Agronomy, Madison Wis. (1987).

A "probe" is an isolated nucleic acid to which is attached a conventional detectable label or reporter molecule, e.g., a radioactive isotope, ligand, chemiluminescent agent, or enzyme. Such a probe is complementary to a strand of a target nucleic acid~~, in the case of the present invention~~, for example to a strand of isolated DNA from corn event TC1507 whether from a corn plant or from a sample that includes DNA from the event. Probes ~~according to the present invention~~ include not only deoxyribonucleic or ribonucleic acids but also polyamides and other probe materials that bind specifically to a target DNA sequence and can be used to detect the presence of that target DNA sequence.

"Primers" are isolated nucleic acids that are annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, then extended along the target DNA strand by a polymerase, e.g., a DNA polymerase. Primer pairs ~~of the present invention~~ refer to their use for amplification of a target nucleic acid sequence, e.g., by the polymerase chain reaction (PCR) or other conventional nucleic-acid amplification methods. "PCR" or "polymerase chain reaction" is a technique used for the amplification of specific DNA segments (see, U.S. Patent Nos. 4,683,195 and 4,800,159~~; herein incorporated by reference~~).

Probes and primers are of sufficient nucleotide length to bind to the target DNA sequence specifically in the hybridization conditions or reaction conditions determined by the operator. This length may be of any length that is of sufficient length to be useful in a detection method of choice. Generally, 11 nucleotides or more in length, preferably 18 nucleotides or more, and more preferably 22 nucleotides or more, are used. Such probes and primers hybridize specifically to a target sequence under high stringency hybridization conditions. Preferably, probes and primers ~~according to the present invention~~ have complete DNA sequence similarity of contiguous nucleotides with the target sequence, although probes differing from the target DNA sequence and that retain the ability to

hybridize to target DNA sequences may be designed by conventional methods. Probes can be used as primers, but are generally designed to bind to the target DNA or RNA and not be used in an amplification process.

Specific primers can be used to amplify an integration fragment to produce an amplicon that can be used as a "specific probe" for identifying event TC1507 in biological samples. When the probe is hybridized with the nucleic acids of a biological sample under conditions which allow for the binding of the probe to the sample, this binding can be detected and thus allow for an indication of the presence of event TC1507 in the biological sample. Such identification of a bound probe has been described in the art. The specific probe is preferably a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the event and preferably also comprises a part of the foreign DNA contiguous therewith. Preferably the specific probe comprises a sequence of at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, and most preferably between 95 and 100% identical (or complementary) to a specific region of the event.

Methods for preparing and using probes and primers are described, for example, in *Molecular Cloning: A Laboratory Manual, 2nd ed.*, vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1989 (hereinafter, "Sambrook *et al.*, 1989"); *Current Protocols in Molecular Biology*, ed. Ausubel *et al.*, Greene Publishing and Wiley-Interscience, New York, 1992 (with periodic updates) (hereinafter, "Ausubel *et al.*, 1992"); and Innis *et al.*, *PCR Protocols: A Guide to Methods and Applications*, Academic Press: San Diego, 1990. PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as the PCR primer analysis tool in Vector NTI version 6 (Informax Inc., Bethesda MD); PrimerSelect (DNASTAR Inc., Madison, WI); and Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Additionally, the sequence can be visually scanned and primers manually identified using guidelines known to one of skill in the art.

A "kit" as used herein refers to a set of reagents for the purpose of performing the method ~~of the invention~~, more particularly, the identification of the event TC1507 in biological samples. The kit ~~of the invention~~ can be used, and its components can be specifically adjusted, for purposes of quality control (e.g. purity of seed lots), detection of event TC1507 in plant material, or material comprising or derived from plant material,

such as but not limited to food or feed products. "Plant material" as used herein refers to material which is obtained or derived from a plant.

Primers and probes based on the flanking DNA and insert sequences disclosed herein can be used to confirm (and, if necessary, to correct) the disclosed sequences by conventional methods, e.g., by re-cloning and sequencing such sequences. The nucleic acid probes and primers ~~of the present invention~~ hybridize under stringent conditions to a target DNA sequence. Any conventional nucleic acid hybridization or amplification method can be used to identify the presence of DNA from a transgenic event in a sample. Nucleic acid molecules or fragments thereof are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are said to be capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure.

A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, 1989, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, a Practical Approach,* IRL Press, Washington, D.C. (1985), departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

In hybridization reactions, specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. The thermal melting point (Tm) is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. For DNA-DNA hybrids, the Tm can be approximated from the equation

of Meinkoth and Wahl (1984) *Anal. Biochem.* 138:267-284: Tm = 81.5°C + 16.6 (log M) + 0.41 (%GC) - 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. Tm is reduced by about 1°C for each 1% of mismatching; thus, Tm, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with >90% identity are sought, the Tm can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the Tm for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the Tm; moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the Tm; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the Tm.

Using the equation, hybridization and wash compositions, and desired Tm, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a Tm of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes*, Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

As used herein, a substantially homologous sequence is a nucleic acid molecule that will specifically hybridize to the complement of the nucleic acid molecule to which it is being compared under high stringency conditions. Appropriate stringency conditions which promote DNA hybridization, for example, 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by a wash of 2X SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for

short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of a destabilizing agent such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. ~~In a preferred embodiment, a~~ A nucleic acid ~~of the present invention will~~ may specifically hybridize to one or more of the nucleic acid molecules unique to the TC1507 event or complements thereof or fragments of either under moderately stringent conditions.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS* 4:11-17; the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math.* 2:482; the homology alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443-453; the search-for-similarity-method of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci.* 85:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA* 87:2264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA* 90:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0); the ALIGN PLUS program (version 3.0, copyright 1997); and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 10 (available from Accelrys, 9685 Scranton Road, San Diego, CA 92121, USA). Alignments using these programs can be performed using the default parameters.

The CLUSTAL program is well described by Higgins and Sharp, *Gene* 73: 237-244 (1988); Higgins and Sharp, *CABIOS* 5: 151-153 (1989); Corpet, *et al., Nucleic Acids Research* 16: 10881-90 (1988); Huang, *et al., Computer Applications in the Biosciences* 8: 155-65 (1992), and Pearson, *et al., Methods in Molecular Biology* 24: 307-

331 (1994). The ALIGN and the ALIGN PLUS programs are based on the algorithm of Myers and Miller (1988) *supra.* The BLAST programs of Altschul *et al.* (1990) *J. Mol. Biol.* 215:403 are based on the algorithm of Karlin and Altschul (1990) *supra.* The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology*, Chapter 19, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). Alignment may also be performed manually by inspection.

To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res.* 25:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) supra. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (e.g., BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See www.ncbi.hlm.nih.gov.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative

substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity.

Regarding the amplification of a target nucleic acid sequence (e.g., by PCR) using a particular amplification primer pair, "stringent conditions" are conditions that permit the primer pair to hybridize only to the target nucleic-acid sequence to which a primer having the corresponding wild-type sequence (or its complement) would bind and preferably to produce a unique amplification product, the amplicon, in a DNA thermal amplification reaction.

The term "specific for (a target sequence)" indicates that a probe or primer hybridizes under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

As used herein, "amplified DNA" or "amplicon" refers to the product of nucleic acid amplification of a target nucleic acid sequence that is part of a nucleic acid template. For example, to determine whether a corn plant resulting from a sexual cross contains transgenic event genomic DNA from the corn plant of the present invention, DNA extracted from the corn plant tissue sample may be subjected to a nucleic acid amplification method using a DNA primer pair that includes a first primer derived from flanking sequence adjacent to the insertion site of inserted heterologous DNA, and a second primer derived from the inserted heterologous DNA to produce an amplicon that is diagnostic for the presence of the event DNA. Alternatively, the second primer may be derived from the flanking sequence. The amplicon is of a length and has a sequence that is also diagnostic for the event. The amplicon may range in length from the combined length

of the primer pairs plus one nucleotide base pair to any length of amplicon producible by a DNA amplification protocol. Alternatively, primer pairs can be derived from flanking sequence on both sides of the inserted DNA so as to produce an amplicon that includes the entire insert nucleotide sequence of the PHI8999A expression construct, see FIG. 1, approximately 6.2 Kb in size. A member of a primer pair derived from the flanking sequence may be located a distance from the inserted DNA sequence, this distance can range from one nucleotide base pair up to the limits of the amplification reaction, or about twenty thousand nucleotide base pairs. The use of the term "amplicon" specifically excludes primer dimers that may be formed in the DNA thermal amplification reaction.

Nucleic acid amplification can be accomplished by any of the various nucleic acid amplification methods known in the art, including the polymerase chain reaction (PCR). A variety of amplification methods are known in the art and are described, inter alia, in U.S. Pat. Nos. 4,683,195 and 4,683,202 and in *PCR Protocols: A Guide to Methods and Applications,* ed. Innis *et al.,* Academic press, San Diego, 1990. PCR amplification methods have been developed to amplify up to 22 Kb of genomic DNA and up to 42 Kb of bacteriophage DNA (Cheng *et al., Proc. Natl. Acad. Sci. USA* 91:5695-5699, 1994). These methods as well as other methods known in the art of DNA amplification may be used ~~in the practice of the present invention~~. It is understood that a number of parameters in a specific PCR protocol may need to be adjusted to specific laboratory conditions and may be slightly modified and yet allow for the collection of similar results. These adjustments will be apparent to a person skilled in the art.

The amplicon produced by these methods may be detected by a plurality of techniques, including, but not limited to, Genetic Bit Analysis (Nikiforov, *et al. Nucleic Acid Res*. 22:4167-4175, 1994) where a DNA oligonucleotide is designed which overlaps both the adjacent flanking DNA sequence and the inserted DNA sequence. The oligonucleotide is immobilized in wells of a microwell plate. Following PCR of the region of interest (using one primer in the inserted sequence and one in the adjacent flanking sequence) a single-stranded PCR product can be hybridized to the immobilized oligonucleotide and serve as a template for a single base extension reaction using a DNA polymerase and labeled ddNTPs specific for the expected next base. Readout may be fluorescent or ELISA-based. A signal indicates presence of the insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Another detection method is the Pyrosequencing technique as described by Winge (*Innov. Pharma. Tech.* 00: 18-24, 2000). In this method an oligonucleotide is designed that overlaps the adjacent DNA and insert DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted sequence and one in the flanking sequence) and incubated in the presence of a DNA polymerase, ATP, sulfurylase, luciferase, apyrase, adenosine 5' phosphosulfate and luciferin. dNTPs are added individually and the incorporation results in a light signal which is measured. A light signal indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single or multi-base extension.

Fluorescence Polarization as described by Chen *et al.*, (*Genome Res.* 9:492-498, 1999) is a method that can be used ~~to detect an amplicon of the present invention~~. Using this method an oligonucleotide is designed which overlaps the flanking and inserted DNA junction. The oligonucleotide is hybridized to a single-stranded PCR product from the region of interest (one primer in the inserted DNA and one in the flanking DNA sequence) and incubated in the presence of a DNA polymerase and a fluorescent-labeled ddNTP. Single base extension results in incorporation of the ddNTP. Incorporation can be measured as a change in polarization using a fluorometer. A change in polarization indicates the presence of the transgene insert/flanking sequence due to successful amplification, hybridization, and single base extension.

Taqman® (PE Applied Biosystems, Foster City, Calif.) is described as a method of detecting and quantifying the presence of a DNA sequence and is fully understood in the instructions provided by the manufacturer. Briefly, a FRET oligonucleotide probe is designed which overlaps the flanking and insert DNA junction. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking genomic sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Hybridization of the FRET probe results in cleavage and release of the fluorescent moiety away from the quenching moiety on the FRET probe. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

Molecular Beacons have been described for use in sequence detection as described in Tyangi *et al.* (*Nature Biotech.* 14:303-308, 1996). Briefly, a FRET oligonucleotide probe is designed that overlaps the flanking and insert DNA junction. The unique

structure of the FRET probe results in it containing secondary structure that keeps the fluorescent and quenching moieties in close proximity. The FRET probe and PCR primers (one primer in the insert DNA sequence and one in the flanking sequence) are cycled in the presence of a thermostable polymerase and dNTPs. Following successful PCR amplification, hybridization of the FRET probe to the target sequence results in the removal of the probe secondary structure and spatial separation of the fluorescent and quenching moieties. A fluorescent signal results. A fluorescent signal indicates the presence of the flanking/transgene insert sequence due to successful amplification and hybridization.

A hybridization reaction using a probe specific to a sequence found within the amplicon is yet another method used to detect the amplicon produced by a PCR reaction.

The present invention is further defined in the following Examples. It should be understood that these Examples, while indicating preferred embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention, in addition to those shown and described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

~~The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.~~

EXAMPLES

Example 1. Transformation of Maize by Particle Bombardment and Regeneration of Transgenic Plants Containing the Cry1F Gene

A DNA molecule of 6.2 Kb, designated PHI8999A (see FIG. 1 and SEQ ID NO:25), which includes a first transgene expression cassette comprising the promoter, 5' untranslated exon, and first intron of the maize ubiquitin (Ubi-1) gene (Christensen *et al.* (1992) Plant Mol. Biol. 18:675-689 and Christensen and Quail (1996) Transgenic Res. 5:213-218) operably connected to a DNA molecule encoding a Bacillus thuringiensis δ-endotoxin identified as Cry1F (U.S. Pat. Nos. 5,188,960 and 6,218,188) operably connected to a DNA molecule comprising a 3' ORF25 transcriptional terminator isolated

from *Agrobacterium tumefaciens* (Barker *et al.* (1983) *Plant Mol. Biol.* 2:335-350), and a second transgene expression cassette comprising a DNA molecule of the cauliflower mosaic virus (CaMV) 35S promoter (Odell J.T. *et al.* (1985) *Nature* 313: 810-812; Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) operably connected to a DNA molecule encoding the selectable marker, phosphinothricin acetyltransferase (PAT) gene (Wohlleben W. *et al.* (1988) *Gene 70*:25-37) operably connected to a DNA molecule comprising a 3' transcriptional terminator from (CaMV) 35S (see Mitsuhara *et al.* (1996) *Plant Cell Physiol.* 37:49-59) was used to transform maize embryo tissue.

B.t. Cry1F maize plants were obtained by microprojectile bombardment using the Biolistics® PDS-1000He particle gun manufactured by Bio-Rad, Hercules, CA; essentially as described by Klein *et al.* (1987) *Nature,* UK 327(6117):70-73. Immature embryos isolated from maize ears, harvested soon after pollination were cultured on callus initiation medium for several days. On the day of transformation, microscopic tungsten particles were coated with purified PHI8999A DNA (SEQ ID NO:25) and accelerated into the cultured embryos, where the insert DNA was incorporated into the cell chromosome. Only insert PHI8999A was used during transformation and no additional plasmid DNA was incorporated into the transformant. After bombardment, embryos were transferred to callus initiation medium containing glufosinate as the selection agent. Individual embryos were kept physically separate during culture, and the majority of explants died on the selective medium.

Those embryos that survived and produced healthy, glufosinate-resistant callus tissue were assigned unique identification codes representing putative transformation events, and continually transferred to fresh selection medium. Plants were regenerated from tissue derived from each unique event and transferred to the greenhouse. Leaf samples were taken for molecular analysis to verify the presence of the transgene by PCR and to confirm expression of the Cry1F protein by ELISA. Plants were then subjected to a whole plant bioassay using European corn borer insects. Positive plants were crossed with inbred lines to obtain seed from the initial transformed plants. A number of lines were evaluated in the field. The TC1507 event was selected from a population of independent transgenic events based on a superior combination of characteristics, including insect resistance and agronomic performance (see Bing JW *et al.* (2000) Efficacy of Cry1F Transgenic Maize, 14th Biennial International Plant Resistance to Insects Workshop, fort Collins, CO~~, herein incorporated by reference~~).

Example 2. Identification of nucleotides comprising the flanking sequence 5' to the transgenic insert DNA in *Bacillus thuringiensis* Cry1F maize line TC1507

To identify a DNA fragment that included sequence 5' to the PHI8999A insert in event TC1507, Spe I restriction enzyme fragments from event TC1507 genomic DNA were size selected on agarose gels, purified, and screened by Southern analysis to confirm hybridization to a Cry1F probe. Following confirmation of hybridization and fragment size, the fragments of interest were cloned into a pBluescript II SK (+)™ cloning vector to prepare an enriched size selected plasmid based genomic DNA library. A probe homologous to a portion of the Cry1F gene was used to screen the plasmid library for positive clones. A positive clone was identified, purified by additional screening, and confirmed to result in a positive signal when hybridized to the Cry1F probe. Nearly 3 Kb of the Spe I fragment contained in the isolated positive clone was sequenced using a primer walking approach. To initiate the first sequencing run, a primer that binds to a known sequence in the cloning vector DNA was designed to sequence a portion of the DNA of interest. A second sequencing run over the same region using another primer oriented in the reverse direction provided second strand coverage. Primer walking was accomplished by repeatedly using sequence data from previous runs to design new primers that were then used to extend the next round of sequencing further into the DNA of interest until the flanking sequence 5' to the inserted transgenic DNA in maize event TC1507 was obtained. Specific sequence information is provided in Example 4.

Example 3. Confirmation of flanking sequence 5' to the *B.t.* Cry1F maize line TC1507 insert

To confirm the 5' flanking sequence of the *B.t.* Cry1F maize line TC1507 insert, PCR primer pairs were designed to obtain overlapping PCR products extending from the 5' flanking region into the full-length PHI8999A transgenic insert. PCR products were successfully amplified from *B.t.* Cry1F maize line TC1507 genomic DNA, isolated, and sequenced for Region 1 through Region 6, shown in Table 1, and confirmed to match the previously determined sequence from the Spe I fragment, described in Example 2. However, the region from bp 2358 to bp 2829, immediately adjacent and 5' to the start of the full-length insert was recalcitrant to PCR amplification and appeared to be larger than the sequence obtained from the Spe I clone described above. The use of primer pairs flanking this region and the Advantage®-GC 2 Polymerase Mix (BD Biosciences Clontech,

Palo Alto, CA) was successful in amplifying PCR products from *B.t.* Cry1F maize line TC1507 genomic DNA for sequencing. The amplification conditions used to produce amplicons with the Advantage®-GC 2 system are shown in Table 10. The DNA primer pairs used to confirm the sequence in the region from bp 2358 to 2829 are those listed in SEQ ID NO:1 and SEQ ID NO:2; and SEQ ID NO:2 and SEQ ID NO:23. Sequence from this region is described in Table 1 (Regions 7a, 7b, 7c, and 8).

Example 4. Event TC1507 5' flanking sequence. A description of each region is provided in Table 1.

Region 1 (SEQ ID NO: 28) Maize genomic (no significant homology)

1 ACTAGTTTCC TAGCCCGCGT CGTGCCCCTA CCCCACCGAC
GTTTATGGAA
51 GGTGCCATTC CACGGTTCTT CGTGGCCGCC CCTAAGGATG
TAAATGGTCG
101 GTAAAATCCG GTAAATTTCC GGTACCGTTT ACCAGATTTT
TCCAGCCGTT
151 TTCGGATTTA TCGGGATATA CAGAAAACGA GACGGAAACG
GAATAGGTTT
201 TTTTTCGAAA ACGGTACGGT AAACGGTGAG ACAAACTTAC
CGTCCGTTTT
251 CGTATTTCTC GGGAAACTCT GGTATATTCC CGTATTTGTC
CCGTATTTTC
301 CCGACCCACG GACCTGCCAA TCAACCATCA GCCAGTCAGC
CCATCCCCAC
351 AGCTATGGCC CATGGGGCCA TGTTGGCCAC ATGCCCACGC
AACGCAAGGC
401 AGTAAGGCTG GCAGCCTGGC ACGCATTGAC GCATGTGGAC
ACACACAGCC
451 GCCGCCTGTT CGTGTTTCTG TGCCGTTGTG CGAGACTGTG
ACTGCGAGTG
501 GCGGAGTCGG CGAACGGCGA GGCGTCTCCG GAGTCTGGAC
TGCGGCTGTG
551 GACAGCGACG CTGTGACGGC GACTCGGCGA AGCCCCAAGC
TACCAAGCCC
601 CCAAGTCCCC ATCCATCTCT GCTTCTCTGG TCATCTCCTT
CCCCTGGTCG
651 ATCTGCAGGC GCCAGACCG

Region 2 (SEQ ID NO: 29) Undescribed maize genomic sequence (complement)

670 G CCGAAGCATC ACGAAACGCA CTAAGACCTC
701 GAAGGAGTCA AACCACTCCT CCGAGGCCTC GGGGGCTACA
CCCGGCGGGT
751 GCGCTCGCGC GCACCCACCG GAACAAAATG TAACCGAGAA
AGGTCGGTCC

801 CCTTGCAAAA AAAGTGCGAC AAAAGCCTCC AAGCGAGTAT
TAACACTCAC
851 TTTGAGGCTC GGGGGCTAC

Region 3 (SEQ ID NO: 30) Fragment of maize Huck-1 retrotransposon

870 T GTCGGGGACC ATAATTAGGG GTACCCCCAA
901 GACTCCTAAT CTCAGCTGGT AACCCCCATC AGCACAAAGC
TGCAAAGGCC
951 TGATGGGTGC GATTAAGTCA AGGCTCGGTC CACTCAAGGG
ACACGATCTC
1001 GCCTCGCCCG AGCCCAGCCT CGGGCAAGGG CGGCCGACCC
CGAGGATTCA
1051 CGTCTCGCCC GAGGGCCCCC TCAAGCGACG GGCACACCTT
CGGCTCGCCC
1101 GAGGCCCATT CTTCGCCGAG AAGCAACCTT GGCCAGATCG
CCACACCGAC
1151 CGACCGTATC GCAGGAGCAT TTAATGCGAG GATCGCCTGA
CACCTTATCC
1201 TGACGCGCGC TCTTCAGTCG ACAGAGCCGA AGTGACCGCA
ATCACTTCGC
1251 CGCTCCACTG ACCGACCTGA CAAGAAGACA GCGCCGCCTG
CGTCGCTCCG
1301 ACTGCTGTGC CACTCGACAG AGTGAGGCTG ACAGCAGCCA
AGTCCGGCCT
1351 CGGGCGCCAT AGGAAGCTCC GCCTCGCCCG ACCCTAGGGC
TCGGACTCGG
1401 CCTCGGCTCC GGAAGACGAC GAACTACGCT TCGCCCGACC
CCAGGGCTTG
1451 GACTCAGCCT CGGCTCCGGA AGACGACGAA TTCCGCCTCG
CCCGACCCCA
1501 GGGCTCGGAC TCGGCCTCGG CTCCAGAAGA CGACGAACTC
CGCCTCGCCC
1551 GACCCCAGGG CTCGGACTCA GCCTCGGCTC CGGAAGACGA
CGAACTCCGC
1601 CTCGCCCGAC CCCAGGGCTC GGACTCAGCC TCGGCCTCAG
ACGATGGTCT
1651 CCGCCTCGCC CGACCCGGGG CTCGGACTCG A

Region 4 (SEQ ID NO: 31) Fragment of *cry*1F gene

1682 CCTTTCTAT CGGACCTTGT
1701 CAGATCCTGT CTTCGTCCGA GGAGGCTTTG GCAATCCTCA
CTATGTACTC
1751 GGTCTTAGGG GAGTGGCCTT TCAACAAACT GGTACGAATC
ACACCCGCAC
1801 ATTCAGGAAC TCCGGGACCA TTGACTCTCT AGATGAGATA
CCACCTCAAG
1851 ACAACAGCGG CGCACCTTGG AATGACTACT CCCATGTGCT
GAATCATGTT

1901 ACCTTTGTGC GCTGGCCAGG TGAGATCTCA GGTTCCGACT
CATGGAGAGC
1951 ACCAATGTTC TCTTGGACGC ATCGTAGCGC TACCCCCACA
AACACCATTG
2001 ATCCAGAGAG AATCAC

Region 5 (SEQ ID NO: 32) Fragment of maize chloroplast *rpo*C2 gene

2017 TCAT TCTTCAAGAA CTGCATATCT TGCCGAGATC
2051 CTCATCCCTA AAGGTACTTG ACAATAGTAT TATTGGAGTC
GATACACAAC
2101 TCACAAAAAA TACAAGAAGT CGACTAGGTG GATTGGTCCG
AGTGAAGAGA
2151 AAAAAAAGCC ATACAGAACT CAAAATCTTT TCCGGAGATA
TTCATTTTCC
2201 TGAAGAGGCG GATAAGATAT TAGGTGGCAG TTTGATACCA
CCAGAAAGAG
2251 AAAAAAAAGA TTCTAAGGAA TCAAAAAAAA GGAAAAATTG
GGTTTATGTT
2301 CAACGGAAAA AATTTCTCAA AAGCAAGGAA AAGTATT

Region 6 (SEQ ID NO: 33) Fragment of maize chloroplast or *ubi*ZM1(2) promoter

2338 GTG GCTATTTATC
2351 TATC

Nucleotides 2355-2358 (CGT) connect Region 6 to Region 7a.

Region 7a (SEQ ID NO: 34) Fragment of *pat* gene

2358 GCA GCTGATATGG CCGCGGTTTG TGATATCGTT AACCATTACA
2401 TTGAGACGTC TACAGTGAAC TTTAGGACAG AGCCACAAAC
ACCACAAGAG
2451 TGGATTGATG ATCTAGAGAG GTTGCAAGAT AGATACCCTT
GGTTGGTTGC
2501 TGAGGTTGAG GGTGTTGTGG CTGGTATTGC TTACGCTGGG
CCCTGGAAGG
2551 CTAGGAAC

Region 7b (SEQ ID NO: 35) Fragment of *pat* gene (complement)

2559 CC TCAACCTCAG CAACCAACCA ATGGTATCTA TCTTGCAACC
2601 TCTCTAGATC ATCAATCCAC TCTTGTGGTG TTTGTGGCTC
TGTCCTAAAG
2651 TTCACTGTAG ACGTCTCAAT GTAATGGTTA ACGATATCAC AAACCG

Region 7c (SEQ ID NO: 36) Fragment of *cry*1F gene (complement)

2697 AGAG
2701 AAGAGGGATC T

Region 8 (SEQ ID NO: 37) Fragment of Polylinker

2712 CGAAGCTTC GGCCGGGGCC CATCGATATC CGCGGGCATG
2751 CCTGCAGTGC AGCGTGACCC GGTCGTGCCC CTCTCTAGAG
ATAATGAGCA
2801 TTGCATGTCT AAGTTATAAA AAATTACCA

Region 9 (SEQ ID NO: 25) Full-length insert of PHI8999A

Example 5. Description of the flanking sequence 5' to the insert in maize event TC1507

In order to more fully describe the event TC1507 5' flanking sequence, homology searching was done against the GenBank public databases (release 122, 2/01) using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, pairwise alignments were performed using AlignX (InforMax Inc., Bethesda, MD) to look for homology between the maize event TC1507 flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The TC1507 5' flanking sequence is numbered with base 1 being the furthest 5' to the insert and base 2830 at the starting point of the full-length PHI8999A transgenic insert (see FIG. 1). The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed.

In most cases, similarity searching with the event TC1507 5' flanking sequence resulted in a match to one unique sequence based on a very high percent identity value. Those sequences are identified in Table 1. In addition, there are two regions in the TC1507 5' DNA flanking sequence with high similarity to more than one known sequence. In regions 870-1681 and 2338-2354, the percent identity scores with both sequence fragments are sufficiently high that a single match (homolog) cannot be determined. The two possible homologs for each of these regions are indicated in Table 1.

Highly similar sequences were identified for all but the first 669 base pairs of sequence. Generally, the results of similarity searching indicate high homology with maize genomic sequences 5' to base 1681. The region from base 1682 to the start of the PHI8999A insert at position 2830 contains some fragments associated with the transformation event.

Table 1. Sequence summary for event TC1507 insert

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
1	1-669	669	N/A[1]	N/A	N/A	No significant homology detected
2	670-869	200	90.5	AF123535	52432-52632 (complement)	Undescribed maize genomic sequence
Region	**Location in SEQ ID NO:24**	**Size bp**	**% Identity**	**Homolog**	**Location in homologous sequence**	**Description**
3	870-1681	812	89.4	AF050439	1-801	Fragment of maize Huck-1 retrotransposon 5' LTR[2]
			86.6	AF050438	1-797	Fragment of maize Huck-1 retro-transposon 3' LTR
4	1682-2016	335	100.0	PHI8999A	3149-3483	Fragment of *cry*1F gene
5	2017-2337	321	100.0	X86563	29429-29749	Fragment of maize chloroplast *rpoC2* gene (RNA polymerase beta-2 subunit)
6	2338-2354	17	100.0	X86563	97643-97659	Fragment of maize chloroplast *trnI* gene (tRNA-Ile)
			82.4	PHI8999A	182-197	Fragment of maize *ubi*ZM1(2) promoter
7a	2358-2558	201	100.0	PHI8999A	5320-5475	Fragment of *pat* gene
7b	2559-2696	138	99	PHI8999A	5336-5518 (complement)	Fragment of *pat* gene
7c	2697-2711	15	100.0	PHI8999A	2544-2558 (complement)	Fragment of *cry*1F gene
8	2712-2829	118	100.0	PHI8999A	36-153	Fragment of polylinker region (bases 36-80) and *ubi*ZM1(2) promoter (bases 81-153)

9	2830-9015	6186	100.0	PHI8999A	11 – 6196	Full-length insert of PHI8999A
10	9016-9565	550	100.0	PHI8999A	3906-4456 (complement)	Inverted ORF25 terminator
11	9566-9693	128	100.0	NC_001666	121851-121978 (complement) & 100759-100886	Fragment of maize chloroplast *rps12* rRNA (23S ribosomal RNA)

Region	Location in SEQ ID NO:24	Size bp	% Identity	Homolog	Location in homologous sequence	Description
12	9696-10087	392	99	NC_001666	17091-17483 (complement)	Fragment of maize chloroplast genome
13	10088-10275	188	99	PHI8999A	5333-5520 (complement)	Fragment of *pat* gene
14	10278-10358	81	100	NC_001666	137122-137202 (complement)	Fragment of maize chloroplast "ORF241" – hypothetical protein gene
15	10359-10612	254	N/A[1]	N/A	N/A	No significant homology detected
16	10613-11361	749	N/A[1]	N/A	N/A	No description available

[1]_ N/A; not applicable
[2]_ LTR; long terminal repeat

Example 6: Confirmation of the presence of Regions 1, 2, and 3 in an unmodified control corn line

PCR analysis was used to determine if Regions 1, 2, and 3 (Table 1) in the 5' flanking region of Event TC1507 are present in an unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Nine different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified control corn line Hi-II (see Armstrong (1994) *The Maize Handbook,* ed. Freeling and Walbot, Springer-Verlag, New York, pp. 663-671, for information on Hi-II) as outlined in Table 2 using the primer sequences shown in Table 3. Two reactions were designed to amplify DNA within Region 1 of the 5' flanking region

from bp 25 to 324 (Reaction A - 300 bp amplicon); and from bp 25 to 480 (Reaction B - 456 bp amplicon). The expected amplicons were present in both the Hi-II unmodified corn line and in maize event TC1507. One PCR primer pair, Reaction C, spanned Region 2 to Region 3 of the 5' flanking region from bp 759 to 1182 (424 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reaction D, spanned Region 1 to Region 3 of the 5' flanking region from bp 415 to 1182 (768 bp amplicon) and again produced PCR products of the expected size in both Hi-II and TC1507. Reactions E and F were designed as specific primer pairs for the *pat* gene region of the full-length insert of PHI8999A in TC1507 and thus an amplicon in the unmodified Hi-II corn line is not expected. The results indicate that both Reactions E and F are specific for a maize line transformed with a *pat* gene region and produce the expected amplicon, whereas no amplicon was produced in the unmodified Hi-II corn line. Reaction G was also designed as a primer pair that would produce an amplicon of 366 bp in the maize event TC1507 and no amplicon in the unmodified Hi-II corn line.

Reactions H and I were designed as specific primer pairs for TC1507 that would span the end of the transgenic insert into the 5' flanking region. In both Reactions H and I, the reverse primer was located in the ubiquitin promoter region of the full-length PHI8999A insert (Region 9 in Table 1) and the forward primer was located in Region 5, the *rpoC2* gene fragment (see Table 1). Reaction H and Reaction I both produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. These results indicate that both Reactions H and I are specific for the TC1507 event.

The PCR results show that the undescribed sequence (Region 1) is present in the unmodified corn line Hi-II and that Regions 1, 2 and 3, are contiguous in the unmodified corn line Hi-II. The DNA sequences amplified in Reactions A, B, C, and D are not unique to the 5' flanking region of maize event TC1507 but are also present in the unmodified corn line Hi-II.

Table 2. PCR reactions for sequence 5' to the PHI8999A insert in maize event TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
A	25 – 324 bp in TC1507 flanking sequence	300	Region 1	Yes	Yes
B	25 – 480 bp in TC1507 flanking sequence	456	Region 1	Yes	Yes

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
	bp in TC1507 flanking sequence		3		
D	415 – 1182 bp in TC1507 5' flanking sequence	768	Region 1 to Region3	Yes	Yes
E Not Unique to TC1507	4750 – 5794 bp in PHI8999A	1045	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes
F Not Unique to TC1507	4827 - 5308 bp in PHI8999A	482	Region 9 (in full-length insert of PHI8999A 35S promoter to *pat* gene)	No	Yes

Reaction	PCR Amplicon Location	Amplicon Size (bp)	Region in TC1507 flanking sequence or PHI8999A insert	Amplicon present In Hi-II	Amplicon present in maize line TC1507
Detects cry1F fragment in 5' flanking region	*cry*1F sequence in 5' flanking sequence and in full-length insert of PHI8999A	366	Spans 335 bp *cry*1F sequence in 5' flanking sequence and same sequence in the full-length insert	No	Yes
H Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3069 bp in Region 9 (full-length insert of PHI8999A)	912	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes
I Unique to TC1507	2158 bp in Region 5 (*rpoC2* gene fragment) to 3001 bp in Region 9 (full-length insert of PHI8999A)	844	Region 5 to Region 9 Unique to Insertion Event [SPANS UNIQUE JUNCTION REGIONS]	No	Yes

Table 3. PCR primers for sequence 5' to the PHI8999A insert in TC1507 and for regions within the full-length insert of PHI8999A in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
A	300	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:11	TTGATTGGCAGGTCCGTGGGTC
B	456	SEQ ID NO:10	CCCCTACCCCACCGACGTTTAT
		SEQ ID NO:12	CACAACGGCACAGAAACACGAA
C	424	SEQ ID NO:13	GCGCACCCACCGGAACAAAATG
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
D	768	SEQ ID NO:15	CCTGGCACGCATTGACGCATGT
		SEQ ID NO:14	TCCTCGCATTAAATGCTCCTGC
E	1045	SEQ ID NO:6	TAGAGGACCTAACAGAACTCGCCGT
		SEQ ID NO:7	GAGCTGGCAACTCAAAATCCCTTT
Reaction	**Amplicon Size (bp)**	**Primer Pair**	**Primer Sequences 5' to 3'**
F	482	SEQ ID NO:8	AAAATCTTCGTCAACATGGTGGAGC

		SEQ ID NO:9	TAATCTCAACTGGTCTCCTCTCCGG
G	366	SEQ ID NO:19	GGCTCGGACTCGACCTTTCTAT
		SEQ ID NO:20	GCAGTTCTTGAAGAATGAGTGA
H	912	SEQ ID NO:1	GTAGTACTATAGATTATATTATTCGT AGAG
		SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
I	844	SEQ ID NO:2	GCCATACAGAACTCAAAATCTTTTCC GGAG
		SEQ ID NO:23	CTTCAAACAAGTGTGACAAA

Example 7. Flanking sequence 3' to inserted transgenic DNA in maize event TC1507

Two separate PCR approaches were used to extend the length of the sequence information 3' to the full-length PHI8999A insert in maize event TC1507. In the first approach PCR primer pairs were designed to amplify a product that spanned the junction between the full-length insert and the inverted ORF25 terminator, see FIG. 1 for a depiction of the inverted ORF25 terminator. A forward primer was located at the end of the full-length PHI8999A insert and a series of reverse primers were located at 100 bp intervals in the inverted sequence. In this manner the length of the inverted fragment present in the maize event TC1507 could be determined within a 100 bp region based on the successful PCR reactions. This method indicated the inverted fragment contained the majority of the ORF25 terminator but no Cry1F sequence. PCR fragments were isolated and sequenced from this region.

In the second approach PCR primers were designed to walk out into the flanking DNA sequence from the inverted ORF25 terminator region as determined in the PCR experiment described above. Genomic DNA isolated from two to three individual plants of event TC1507 and an unmodified control corn line was digested with various restriction enzymes and then ligated to adaptors specific for the restriction enzyme used for digestion (Universal Genome Walker™ Kit, Clontech Laboratories, Inc. and Devon *et al.* (1995) *Nucleic Acids Res.* 23:1644-1645). Primary PCR was carried out using an ORF25 terminator specific primer and a primer homologous to the adaptor sequence ligated onto the digested DNA. In order to increase the specificity of the reaction a nested secondary PCR was performed again with another ORF25 terminator specific primer and a secondary primer homologous to the adaptor sequence with the secondary primers being internal to the respective primers used in the primary PCR. Products produced by the nested PCR

were analyzed by agarose gel electrophoresis and fragments unique to TC1507 DNA samples were isolated and sequenced. Fragments were amplified from both the ORF25 terminator contained within the full-length insert and from the targeted (inverted) ORF25 terminator on the 3' end of the full-length PHI8999A insert. Fragments from the full-length insert were of a predicted size based on the knowledge of the restriction enzyme sites located in the full-length insert. Fragments produced from the 3' inverted ORF25 terminator appeared as fragments of unexpected size. Sequence analysis of amplified fragments from the 3' inverted ORF25 terminator resulted in flanking DNA sequence of 1043 bp. Resultant sequence from the above series of genome walking experiments was used to design additional primers to walk further out from the insert into the bordering maize genome with a final 3' flanking sequence, of 2346 bp.

In order to describe the TC1507 3' flanking sequence, homology searching was done against the GenBank public databases using the Basic Local Alignment Search Tool (BLAST). The BLAST program performs sequence similarity searching and is particularly useful for identifying homologs to an unknown sequence. In addition to searching the public databases, alignments were performed using SeqMan 4.05™, Martinez and Needleman-Wunsch alignment algorithms (DNASTAR Inc.) to look for homology between the TC1507 3' flanking sequence and the PHI8999A transgenic insert. The results of these homology searches are presented in Table 1. The percent identity values indicate the percentage of identical matches across the length of the sequences analyzed. The results of similarity searching for the 3' flanking sequence indicate high homology with three regions of maize chloroplast DNA, a 188 bp fragment of the *pat* gene, and 254 bp of DNA (Region 15, Table 1) with no significant homology. An additional 749 bp (Region 16) beyond Region 15 (see Table 1) was also sequenced. No similarity searching results are available for Region 16.

PCR analysis on control and TC1507 genomic DNA determined that the 254 bp sequence (Region 15, fragment of maize chloroplast "ORF241") is present in the maize genome. The DNA sequence of Region 15 in the 3' flanking region is not unique to the 3' flanking region of maize event TC1507 but is also present in the unmodified control corn line. The TC1507 3' flanking sequence is presented in Example 8 and diagrammed in FIG. 1.

Example 8. Sequence of the region 3' to the end of the full-length insert DNA in maize event TC1507. A description of each region is in Table 1.

Region 10 (SEQ ID NO: 38) Fragment of ORF25 Terminator (complement)

9016 CTCAC TCCGCTTGAT CTTGGCAAAG ATATTTGACG
9051 CATTTATTAG TATGTGTTAA TTTTCATTTG CAGTGCAGTA
TTTTCTATTC
9101 GATCTTTATG TAATTCGTTA CAATTAATAA ATATTCAAAT
CAGATTATTG
9151 ACTGTCATTT GTATCAAATC GTGTTTAATG GATATTTTTA
TTATAATATT
9201 GATGATATCT CAATCAAAAC GTAGATAATA ATAATATTTA
TTTAATATTT
9251 TTGCGTCGCA CAGTGAAAAT CTATATGAGA TTACAAAATA
CCGACAACAT
9301 TATTTAAGAA ACATAGACAT TAACCCTGAG ACTGTTGGAC
ATCAACGGGT
9351 AGATTCCTTC ATGCATAGCA CCTCATTCTT GGGGACAAAA
GCACGGTTTG
9401 GCCGTTCCAT TGCTGCACGA ACGAGCTTTG CTATATCCTC
GGGTTGGATC
9451 ATCTCATCAG GTCCAATCAA ATTTGTCCAA GAACTCATGT
TAGTCGCAAC
9501 GAAACCGGGG CATATGTCGG GTATCTCGAG CTCGCGAAAG
CTTGGCTGCA
9551 GGTCGACGGA TCCTT

Region 11 (SEQ ID NO: 39) Fragment of maize chloroplast *rps12* rRNA gene (complement)

9566 CAACA AAAGGGTACC TGTACCCGAA ACCGACACAG
9601 GTGGGTAGGT AGAGAATACC TAGGGGCGCG AGACAACTCT
CTCTAAGGAA
9651 CTCGGCAAAA TAGCCCCGTA ACTTCGGGAG AAGGGGTGCC CCC

Nucleotides 9694-9695 (CG) connect Region 11 to Region 12.

Region 12 (SEQ ID NO: 40) Fragment of maize chloroplast genome

9696 CTAAC
9701 AATAAACGAA TACGGTTTAT GTATGGATTC CGGTAAAATA
CCGGTACTCG
9751 ATTTCATAAG AGTCGAATAG GAAGTTAAGA TGAGGGTGGT
ATCATCATAA
9801 AAATGGAGTA GTATCCTAAA TTATACTAAT CCACGTATGA
TATGTATGCC
9851 TTTCCTTATC AACCGGAAGT AGTGCAAAAA AAATTCTATA
CTGCACTGCT

9901 CTCTTTTTAC TGAGAAATGC AAAAAAATAA AAGTGAAGTA
AGGGTGCCCC
9951 ATAGATATTT GATCTTGCCT CCTGTCCCCC CCCCCCTTTT
TTCATCAAAA
10001 ATTTCCATGA AAAAAGAAAA GATGAATTTG TCCATTCATT
GAACCCTAGT
10051 TCGGGACTGA CGGGGCTCGA ACCCGCAGCT TCCGCCT

Region 13 (SEQ ID NO: 41) Fragment of *pat* gene (complement)

10088 GTT CCTAGCCTTC
10101 CAGGGCCCAG CGTAAGCAAT ACCAGCCACA GCACCCTCAA
CCTCAGCAAC
10151 CAACCAAGGG TATCTATCTT GCAACCTCTC TAGATCATCA
ATCCACTCTT
10201 GTGGTGTTTG TGGCTCTGTC CTAAAGTTCA CTGTAGACGT
CTCAATGTAA
10251 TGGTTAACGA TATCACAAAC CGCGG

Nucleotides 10276-10277 (AA) connect Region 13 to Region 14.

Region 14 (SEQ ID NO: 42) Fragment of maize chloroplast ORF241 (complement)

10278 CAC AAGAACGAAA GCACCTTTTC
10301 ATTCTTTCAT ATACTAGGGG TTTTTACTTG GAAAAGACAA
TGTTCCATAC
10351 TAAAGGAT

Region 15 (SEQ ID NO: 43) Maize genomic (no significant homology)

10359 AG CTGCAGAAGC CGCCACCGTC TTGAGGACCT TCCGGGGAGC
10401 CAGACCGGTC GAACCGTGCC TCCACTTGCT AAGGAGAAAG
GGAAAATCAG
10451 GGCCAGGACA TACGAAGGAG GAGCCAGAAC GAAGATATCC
TAAGATACTT
10501 ACTCGCTCCG GGCCATGATC AATCATGCCT GTGGGGAGGT
CTCTCGCACC
10551 TCGATCCATG AAGGTACCAC CGAGGTCTGC CCCGCCGCCG
GCTTCGGTAC
10601 CGTCCTCGCC TT

Region 16 (SEQ ID NO: 44) Maize genomic

10613 GGGCGCCC GAGGCACCCG GGGGATGGAC TGCCCAGGCG
10651 CAGCCACGAC GACCCAAGGA TCACCCTCCT GCGCAGTCGG
CACGAGCAAT
10701 AGTTCTCGGG GAACAGGCAG CTTGGCCTGA CTCCCCGGGG
TCACCTCAAC
10751 TACCTCGGCC GAGGGGTCAA GTACCCCCTC AGTCCGCCCC
CGCTCTTCGG

10801 ACCGGGACCC CGACGTCCCG GCCCCGGATA CCGACGGCAC
CAGCCCGCTC
10851 GGGGGCTGGC TTGACGACCC CTGGCCCAGC CTCAGATCTG
GGCTGAGGCC
10901 GAGGCAGGCG GCCATGTCGT CGTCTTCATC ATCGTCTTCA
TCATCGTCGT
10951 CGTCATCAGG CGTCTCCGGC GACGGCTCCC TTGGGAGCCC
CTCCCTCTCC
11001 TGCCGACGAC GAAGCCTTTC CAAGGCATCC CGAGCCCACG
TCCGCTCGTG
11051 GGCCCGAGCC TTCTTTGCGT CCTTCTTCTC CTTCCTCTTC
TCCGCGGTGA
11101 CCCTCCGCGC AGCTCGGTCC ACCGCATCCT CCGGGACTGG
TGGCAGGGAA
11151 GGCTTGTGAT GCCCTACCTC CTGGAGACAG ACGAAAAGTC
TCAGCTATGA
11201 GAACCGAGGG CAATCTGACG CAAGAAGGAA GAAGGAGCGG
ATACTCACCA
11251 GAGACACGCA CCCGCGATCG GGACGCATTA AGGGCTGGGA
AAAAGTGCCG
11301 GCCTCTAATT TCGCTACCGT GCCGTCCACC CACCTGTGGA
GGTCATCGAT
11351 GGGAAGGGGA A

Example 9. Confirmation of the presence of Region 15 in the unmodified control corn line

PCR analysis was used to determine if the undescribed region of sequence on the end of the 3' flanking sequence (Region 15 in Table 1) is present in the unmodified control corn line used for transformation to produce maize event TC1507 and thus represents a border with corn genomic DNA. Successful PCR amplification of Region 15 in both maize line TC1507 and the unmodified Hi-II control corn line revealed that Region 15 was indeed present in corn genomic DNA. Five different PCR analyses were carried out on genomic DNA prepared from TC1507 and the unmodified Hi-II control corn line as outlined in Table 7 below using the primer sequences shown in Table 8. Three reactions were designed to amplify DNA within Region 15 of the 3' flanking region; Reaction L – producing a 175 bp amplicon, Reaction M – producing a 134 bp amplicon, and Reaction N – producing a 107 bp amplicon. The expected amplicons were present in both the unmodified control corn line and in maize line TC1507. Reactions J and K were designed as specific primer pairs for TC1507 that would span the end of the insert into the 3' flanking region. In Reaction J, the forward primer was located in the *pat* gene fragment

on the 3' end of the full-length PHI8999A insert (Region 13 in Table 1) and the reverse primer was located in the undefined Region 15. In Reaction K the forward primer was located in the chloroplast hypothetical protein gene on the 3' end of the full-length insert (Region 14 in Table 1) and the reverse primer was located in the undefined Region 15. Both Reaction J and Reaction K produced an amplicon in maize line TC1507 and did not produce an amplicon in the unmodified control corn line. The results indicate that both Reactions J and K are specific for the TC1507 event.

The PCR results indicate that the undescribed sequence (Region 15) of the 3' flanking sequence of TC1507 is also present in genomic DNA isolated from the unmodified Hi-II control corn line. The DNA sequences amplified in Reactions L, M, and N are not unique to the 3' flanking region of TC1507 but are also present in the unmodified control corn line.

Table 7. PCR reactions for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Region in TC1507 3' flanking sequence	Amplicon present in Control	Amplicon present in maize line TC1507
J	342	Region 13 (*pat* gene fragment) to Region 15	No	Yes
K	252	Region 14 (chloroplast gene) to Region 15	No	Yes
L	175	Region 15	Yes	Yes
M	134	Region 15	Yes	Yes
N	107	Region 15	Yes	Yes

Table 8. PCR primers for sequence 3' to the PHI8999A insert in maize event TC1507

Reaction	Amplicon Size (bp)	Primer Pair	Primer Sequences 5' to 3'
J	342	SEQ ID NO:3	TGTGGTGTTTGTGGCTCTGTCCTAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTGATC
K	252	SEQ ID NO:4	AGCACCTTTTCATTCTTTCATATAC

		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
L	175	SEQ ID NO:16	AAGCCGCCACCGTCTTGAGGAC CTT
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
M	134	SEQ ID NO:17	GTCGAACCGTGCCTCCACTTGC TAA
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC
N	107	SEQ ID NO:18	AGAAAGGGAAAATCAGGGCCA GGAC
		SEQ ID NO:5	GACCTCCCCACAGGCATGATTG ATC

Example 10. PCR Primers

DNA event specific primer pairs were used to produce an amplicon diagnostic for TC1507. These event primer pairs include, but are not limited to, SEQ ID NO: 1 and SEQ ID NO: 2; SEQ ID NO: 2 and SEQ ID NO: 23; SEQ ID NO: 3 and SEQ ID NO: 5; and SEQ ID NO: 4 and SEQ ID NO: 5. In addition to these primer pairs, any primer pair derived from SEQ ID NO: 26 and SEQ ID NO: 27 that when used in a DNA amplification reaction produces a DNA amplicon diagnostic for TC1507 is disclosed~~an aspect of the present invention~~. The amplification conditions for this analysis are illustrated in Table 9, however, any modification of these methods that use DNA primers or complements thereof to produce an amplicon DNA molecule diagnostic for TC1507 is within the ordinary skill of the art. The preferred amplification conditions for reactions utilizing the PCR primers identified in SEQ ID NOS: 1, 2, and 23 are illustrated in Table 10. In addition, control primer pairs, which include SEQ ID NOS: 10 and 11; SEQ ID NOS: 10 and 12; SEQ ID NOS: 13 and 14; SEQ ID NOS: 14 and 15; SEQ ID NOS: 5 and 16; SEQ ID NOS: 5 and 17; and SEQ ID NOS: 5 and 18; for amplification of an endogenous corn gene are included as internal standards for the reaction conditions. Also included are primer pairs that will produce an amplicon in transgenic events containing a *pat* gene (SEQ ID NOS: 6 and 7; SEQ ID NOS: 8 and 9), and a primer pair that will produce an amplicon in transgenic events containing a *cry*1F gene (SEQ ID NOS: 19 and 20).

The analysis of plant tissue DNA extracts to test for the presence of the TC1507 event should include a positive tissue DNA extract control (a DNA sample known to contain the transgenic sequences). A successful amplification of the positive control

demonstrates that the PCR was run under conditions which allow for the amplification of target sequences. A negative, or wild-type, DNA extract control in which the template DNA provided is either genomic DNA prepared from a non-transgenic plant, or is a non-TC1507 transgenic plant, should also be included. Additionally a negative control that contains no template corn DNA extract will be a useful gauge of the reagents and conditions used in the PCR protocol.

Additional DNA primer molecules of sufficient length can be selected from SEQ ID NO: 26 and SEQ ID NO: 27 by those skilled in the art of DNA amplification methods, and conditions optimized for the production of an amplicon that may differ from the methods shown in Table 9 or Table 10 but result in an amplicon diagnostic for event TC1507. ~~The use of these DNA primer sequences with modifications to the methods shown in Table 9 and Table 10 are within the scope of the invention.~~ The amplicon wherein at least one DNA primer molecule of sufficient length derived from SEQ ID NO: 26 and SEQ ID NO: 27 that is diagnostic for event TC1507 is disclosed~~an aspect of the invention~~. The amplicon wherein at least one DNA primer of sufficient length derived from any of the genetic elements of PHI8999A that is diagnostic for event TC1507 is disclosed~~an aspect of the invention~~. The assay for the TC1507 amplicon can be performed by using a Stratagene Robocycler, MJ Engine, Perkin-Elmer 9700, or Eppendorf Mastercycler Gradient thermocycler, or by methods and apparatus known to those skilled in the art.

Table 9. PCR Conditions:

Conditions:

Kit used:	Perkin-Elmer AmpliTAQ Gold kit
Volume	Component
5 µl	template (10 ng/µl)
4 µl	2 µl each primer (10 µM)
2 µl	10X PCR Gold Buffer
2 µl	25 mM $MgCl_2$
2 µl	50X dNTP's (10 mM)
0.1 µl	Amplitaq Gold Polymerase

4.9 µl	H_2O
20 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

9 min 92°C
30 cycles:
94°C 30 sec
60°C 30 sec
72°C 1 min
7 min 72°C
Hold 4°C

Table 10. PCR Conditions used with the Advantage®-GC 2 Polymerase Mix:

Conditions:

Kit used:	Advantage®-GC 2 Polymerase Mix
Volume	Component
5 µl	template (10 ng/µl)
5 µl	2.5 µl each primer (10 µM)
10 µl	5x GC2 Buffer
10 µl	GC melt (1.0 M final conc.)
1.5 µl	50X dNTP's (10 mM)
1.0 µl	Advantage GC2 Polymerase
17.5 µl	H_2O
50 µl	Total

Cycling Parameters
GeneAmp® PCR System 9700

5 min 94°C
35 cycles:
94°C 1 min
60°C 2 min
72°C 3 min
7 min 72°C
Hold 4°C

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. ~~We claim all modifications that are within the spirit and scope of the appended claims.~~

~~All publications and published patent documents cited in this specification are incorporated herein by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.~~

~~Further Embodiments of the Invention are as follows:~~

~~1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21.~~

~~2. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:22.~~

~~3. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:24.~~

~~4. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:26.~~

~~5. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:27.~~

~~6. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising at least a first primer, which recognizes a sequence within SEQ ID NO:21 or within SEQ ID NO:22.~~

~~7. The kit of item 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.~~

~~8. The kit of item 6, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.~~

~~9. The kit of item 6, wherein the first primer recognizes a sequence within SEQ ID NO:21.~~

~~10. The kit of item 6, wherein the first primer recognizes a sequence within SEQ ID NO:22.~~

~~11. The kit of item 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.~~

~~12. The kit of item 7, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.~~

~~13. The kit of item 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.~~

~~14. The kit of item 8, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.~~

15. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 26.

16. A DNA detection kit specific for junction DNA of maize event TC1507 and its progeny comprising at least one DNA molecule of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method, that is homologous or complementary to SEQ ID NO: 27.

17. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with SEQ ID NO:21 and SEQ ID NO:25, contiguous therewith.

18. A kit for identifying event TC1507 in a biological sample, said kit comprising a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

19. A DNA detection kit comprising: at least one DNA molecule of a sufficient length of contiguous nucleotides homologous or complementary to SEQ ID NO:26 or SEQ ID NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny.

20. A DNA construct comprising: a first and second expression cassette, wherein said first expression cassette in operable linkage comprises

(a) a maize ubiquitin promoter;

(b) a 5' untranslated exon of a maize ubiquitin gene;

(c) a maize ubiquitin intron;

(d) a Cry1F encoding DNA molecule; and

(e) a 3' ORF25 transcriptional terminator; and

said second expression cassette comprising in operable linkage

(i) a CaMV 35S promoter;

(ii) a *pat* encoding DNA molecule; and

(iii) a 3' transcriptional terminator from (CaMV) 35S.

21. A plant comprising the DNA construct of item 20.

22. A plant of item 21, wherein said plant is a corn plant.

~~23. A method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22.~~

~~24. The method of item 23, further comprising amplifying a DNA fragment from a nucleic acid present in said biological sample using a polymerase chain reaction with at least two primers, wherein said first primer recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, and a second primer recognizes a sequence within SEQ ID NO:22 or SEQ ID NO: 25.~~

~~25. The method of item 24, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.~~

~~26. The method of item 23, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.~~

~~27. The method of item 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively.~~

~~28. The method of item 25, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.~~

~~29. The method of item 26, wherein said first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively.~~

~~30. The method of item 26, wherein said first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5 respectively.~~

~~31. The method of item 27, comprising amplifying a fragment of about 912 bp using a TC1507 PCR identification protocol.~~

~~32. The method of item 28, comprising amplifying a fragment of about 844 bp using a TC1507 PCR identification protocol.~~

~~33. The method of item 29, comprising amplifying a fragment of about 342 bp using a TC1507 PCR identification protocol.~~

~~34. The method of item 30, comprising amplifying a fragment of about 252 bp using a TC1507 PCR identification protocol.~~

~~35. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:~~

~~(a) extracting a DNA sample from said biological sample;~~

~~(a) providing DNA primer molecules SEQ ID NO:1 and SEQ ID NO:2 ;~~

(a) providing DNA amplification reaction conditions;

(a) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(a) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

36. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(a) providing DNA primer molecules SEQ ID NO:2 and SEQ ID NO:23 ;

(a) providing DNA amplification reaction conditions;

(a) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(a) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

37. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(a) providing DNA primer molecules SEQ ID NO:3 and SEQ ID NO:5 ;

(a) providing DNA amplification reaction conditions;

(a) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(a) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

38. A method of detecting the presence of maize event TC1507 or progeny thereof in a biological sample, comprising:

(a) extracting a DNA sample from said biological sample;

(b) providing DNA primer molecules SEQ ID NO:4 and SEQ ID NO:5 ;

(b) providing DNA amplification reaction conditions;

(b) performing said DNA amplification reaction, thereby producing a DNA amplicon molecule; and

(b) detecting said DNA amplicon molecule, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates the presence of maize event TC1507.

39. An isolated DNA molecule comprising the amplicon produced by the method of item 35.

40. An isolated DNA molecule comprising the amplicon produced by the method of item 36.

41. An isolated DNA molecule comprising the amplicon produced by the method of item 37.

42. An isolated DNA molecule comprising the amplicon produced by the method of item 38.

43. A method of detecting the presence of DNA corresponding to the TC1507 event in a sample, the method comprising:

a. contacting the sample comprising maize DNA with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from maize event TC1507 and does not hybridize under said stringent hybridization conditions with a non-TC1507 maize plant DNA;

b. subjecting the sample and probe to stringent hybridization conditions; and

c. detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the TC1507 event.

44. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 or its complement.

45. An isolated DNA nucleotide primer sequence of item 44 comprising a sequence selected from the group consisting of SEQ ID NO:1, 2, 3, 4, 5 and 23 or its complement.

46. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:26 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.

47. A pair of DNA molecules comprising: a first DNA molecule and a second DNA molecule, wherein the DNA molecules are of a sufficient length of contiguous nucleotides of SEQ ID NO:27 or its complement to function as DNA primers or probes diagnostic for DNA extracted from a TC1507 corn plant or progeny thereof.

48. An isolated DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

49. A method for confirming seed purity, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22, in a seed sample.

50. A method for screening seeds for the presence of event TC1507, comprising detection of a TC1507 specific region with a specific primer or probe which specifically recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot.

51. An insect resistant corn plant, or parts thereof, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

52. A descent plant of the insect resistant corn plant of item 51 wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome.

53. Seed of a plant of items 51 or 52.

54. A method of producing an insect resistant corn plant comprising breeding with a plant of items 51 or 52, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

55. An isolated DNA sequence comprising a sequence selected from the group consisting of DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

56. A pair of isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will produce an amplicon diagnostic for event TC1507.

57. The pair of isolated DNA sequences of item 56 wherein each is chosen from SEQ ID NO:26.

58. The pair of isolated DNA sequences of item 56 wherein each is chosen from SEQ ID NO:27.

59. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) selecting a primer pair each comprising at least ten nucleotides from SEQ ID NO:26 or SEQ ID NO:27 wherein each member of the pair is on opposite sides of a sequence diagnostic for said TC1507 event insertion;

(a) contacting a sample of said corn tissue with said primer pair;

(a) performing DNA amplification and analyzing for an amplicons.

60. The method of item 59 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

61. A method of detecting the presence of the TC1507 event insertion in corn tissue comprising:

(a) contacting a sample of said corn tissue with a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof;

(a) subjecting said sample and probe to stringent hybridization conditions; and

(a) analyzing for hybridization of the probe.

62. A DNA detection kit comprising a polynucleotide probe that hybridizes under stringent hybridization conditions with one or more DNA sequences selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.

~~63. A DNA detection kit comprising a primer pair each comprising at least 10 nucleotides from SEQ ID NO:26 and SEQ ID NO:27, wherein each is on opposite sides of a sequence diagnostic for the TC1507 event insertion.~~

~~64. The DNA detection kit of item 61 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.~~

~~65. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.~~

~~66. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.~~

CLAIMS

1. An insect resistant corn plant or parts thereof, or corn seed, comprising the nucleotide sequence of SEQ ID NO: 24.

2. The plant or seed of claim 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising ~~event TC1507 or~~ SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

~~3. An insect resistant corn plant or parts thereof, or a descent plant of said corn plant, or corn seed, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof forms part of the genome of the plant or seed.~~

~~4. An insect resistant corn plant obtainable by breeding with a plant of claim 3, and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 and 57 and complements thereof.~~

~~5. A kit for identifying event TC1507 of SEQ ID NO: 24 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.~~

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	6379113
Application number	EP15168051.9
Date of receipt	01 June 2018
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file

Documents submitted	package-data.xml epf1038.pdf (1 p.) CLMS-1.PDF\Amended claims (clean) Jun18 N96314A-EP AXB KKT.PDF (1 p.) DESC-1.PDF\Amended descr (clean) Jun18 N96314A-EP AXB KKT.PDF (39 p.)	ep-sfd-request.xml EXRE3-1.PDF\Response N96314A-EP AXB KKT.PDF (2 p.) CLMS-HWA-1.pdf\Amended claims (tracked) Jun18 N96314A-EP AXB KKT.pdf (1 p.) DESC-HWA-1.pdf\Amended desc (tracked) Jun18 N96314A-EP AXB KKT.pdf (54 p.)

Submitted by	CN=Kapil Tuladhar 55592
Method of submission	Online
Date and time receipt generated	01 June 2018, 12:52 (CEST)
Message Digest	50:7B:D7:BC:FB:58:92:CD:5A:B0:C0:58:51:DD:02:13:17:12:A6:49

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82,

OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

Application No. : 15 168 051.9

Consultation by telephone with the applicant / representative

Despatch with a time limit of 4 month(s)

Participants

Applicant: Dow AgroSciences, LLC

Representative: Tuladhar, Kapil

Member(s) of the Examining Division: Helliot, Bertrand

Result of consultation

See Separate Sheet

28.06.2018
. . . . . .
Date

Helliot, Bertrand
. . . . . .
Examiner

Enclosure(s):

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME-UNI

Formalities Officer
Name: Vasilakis, Stylianos
Tel: +49 89 2399 - 8554
or call
+31 (0)70 340 45 00

Substantive Examiner
Name: Helliot, Bertrand
Tel: +49 89 2399 - 7793

Application No.	Ref.	Date
15 168 051.9 - 1118	N96314A-EP	04.07.2018
Applicant: Dow AgroSciences, LLC, et al		

Communication pursuant to Article 94(3) EPC

Examination of the above application has revealed that, for the reasons enclosed herewith which - at least partly - reflect the result of consultation by telephone on 28.06.2018, it does not meet the requirements of the European Patent Convention. If the deficiencies indicated are not rectified the application may be refused pursuant to Article 97(2) EPC.

You are invited to file your observations and insofar as the deficiencies are such as to be rectifiable, to remedy the indicated deficiencies within a period

of 4 months

from notification of this invitation.

The time limit is calculated in accordance with the provisions of Rule 126(2), 131(2) and (4) EPC. One set of amendments to the description, claims and drawings is to be filed within the said period on separate sheets (R. 50(1) EPC).

If filing amendments, you must identify them and indicate the basis for them in the application as filed. Failure to meet either requirement may lead to a communication from the Examining Division requesting that you correct this deficiency (R. 137(4) EPC).

Failure to reply to this invitation in due time will result in the European application being deemed to be withdrawn (Art. 94(4) EPC).

Registered letter

Helliot, Bertrand
For the Examining Division

Enclosure(s): Copy of result of consultation (Form 2036)

Registered letter
EPO Form 2049B 11.17 TRI

The Applicant (k. Tuladhar) was informed that present claim 1 does not meet the requirements of Art. 56 EPC as the claimed subject-matter is not limited to the insect resistant corn plant comprising the event TC1507 characterised by the nucleotide sequence SEQ ID N° 24. The Examining Division doubts that the resistance to insect can be obtained by insering the sequence SEQ ID N° 24 in any region of the genome.

Moreover, in the case said objection would be overcome, the Applicant is informed that the claimed subject-matter would violate Art. 83 EPC. Thus, the claimed subject-matter includes a particular event resulting from a random method and having one surprising and advantageous property, i.e. insect resistance.

According to the decision T657/10, the disclosure has to enable the skilled person to obtain the particular product resulting from the TC1507. In view of your letter dated 16.10.2014, wherein you stated that "in view of the section of Pioneer agreement headed “I agree” it is clear that the grower is only permitted to use the seed to raise a commercial crop in a single season and not for any other purpose. In particular the grower is prohibited from transmitting the seed onwards to third parties for planting, breeding, seed production or research purposes. The grower is also prohibited from using the seeds for such purposes", these requirements are not fulfiled.

Finally, in the case this objection would be overcome by filing evidences that the claimed subject-matter was indeed available to the public, the Applicant is informed that the claimed subject-matter would violate Art. 54 EPC.

J A · K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

13 November 2018

Dear Sirs

European Patent Application No. 15168051.9
DOW AGROSCIENCES LLC *et al.*
Our Ref: N96314A-EP AXB/KKT/mhm

I refer to your Communication of 4 July 2018 and request a two month extension to the period for response, taking the total response period to six months. The Applicant requires more time to consider the Examiner's objections. Please confirm in due course that this extension has been granted.

Yours faithfully

Electronically Signed
DR KAPIL TULADHAR
ASSOCIATION NO. 811

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • PARIS • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Request for extension of time limit during examination procedure	Request examination report extension - N96314A-EP AXB KKT.pdf	EXRE92-1.pdf

	Payment	
1	Method of payment	Not specified

Signatures

Place:	**Oxford, GB**
Date:	**13 November 2018**
Signed by:	**Kapil Tuladhar 55592**
Association:	**J A Kemp**
Representative name:	**Kapil TULADHAR**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	6905540
Application number	EP15168051.9
Date of receipt	13 November 2018
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml EXRE92-1.pdf\Request examination report extension - N96314A-EP AXB KKT.pdf (1 p.)
Submitted by	CN=Kapil Tuladhar 55592
Method of submission	Online
Date and time receipt generated	13 November 2018, 19:10 (CET)
Message Digest	34:CE:6D:BC:2D:37:7D:E7:0E:85:44:F7:FC:EC:4A:06:F9:BB:0B:F5

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
19.11.18

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Extension of time limit pursuant to Rule 132(2) EPC

Examination procedure

With reference to your request, the time limit for replying to the communication pursuant to Article 94(3) EPC dated 04.07.18 has been extended

by 2 months

to a total of 6 months

from the date of notification of the above-mentioned communication.

Please note: To the extent that your request exceeded the above extension, your request has been refused.

Note
The granting of extensions to time limits is governed by the Implementing Regulations to the EPC and the Guidelines for Examination in the EPO, E-VIII, 1.6. A request for extension, which would result in the total period set exceeding six months, must be reasoned and supported by evidence.

If no reply to the communication is received in due time, the European patent application will be deemed to be withdrawn (Art. 94(4) EPC).

Important information in case of a PACE request
If a PACE request has been validly filed for the application, the applicant is herewith informed that with the grant of the request for extension of time limit the application is removed from the PACE programme. A second PACE request for that application during the same stage of the procedure will not be processed (see OJ EPO 2015, A93, point 4).

For the Examining Division



J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

4 January 2019

Dear Sirs

European Patent Application No. 15168051.9
DOW AGROSCIENCES LLC *et al.*
Our Ref: N96314A-EP AXB/KKT/mqr

In response to the Examination Report dated 4 July 2018, please replace the claims at present on file with the new claims provided. A marked-up version of the claims showing the amendments being made is also provided for the Examining Division's reference.

Amendments

Claim 1 has been amended to refer to "*TC1507 corn plant or parts thereof characterised by SEQ ID NO: 24, or TC1507 corn seed characterised by SEQ ID NO: 24*", as requested by the Examiner. Thus, it is now clear that the nucleotide sequence of SEQ ID NO: 24 must be inserted into the corn genome at the insertion site defined by the flanking sequences of SEQ ID NO: 24. Basis for this amendment can be found, for example, at page 9, lines 5-6 of the original application.

Comments

Claim 1 explicitly requires that the corn plant, parts thereof, or corn seed, is "*insect resistant*". Further, SEQ ID NO: 24 includes both the 5' and 3' flanking sequences necessarily generated by the introduction of exogenous genetic material at a specific site within the corn genome. Therefore, it is submitted that the Examining Division's interpretation that SEQ ID NO: 24 may in principle be inserted anywhere is the corn genome is not consistent with the context of the invention. When read with a mind willing to understand, as required by Decision T190/99, it is clear that the SEQ ID NO: 24 must define the specific insertion defining the TC1507 event.

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • PARIS • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

Nevertheless, to advance prosecution, the Examiner's suggestion has been taken into account and claim 1 amended such that it is explicitly characterised by the TC1507 event (i.e., the insertion at a specific location of the corn genome). The specific insertion defining the TC1507 event is neither taught nor suggested by the cited documents. Accordingly, the amended claims meet the requirements of Article 56 EPC.

The Examining Division goes on to cite Decision T657/10, together with our letter dated 16 October 2014 filed on the parent application. However, the statement in the letter of 16 October 2014 regarding the "bag tag" language (i.e., the fact that a grower had to agree not to save seed or propagate seed for sale), does not by any means suggest that the application was insufficient at the priority date. All it shows is that the invention is novel because the information required to repeat it was not publicly available at the time. For sufficiency, the question is whether the additional information present in the specification renders the invention repeatable.
It is also submitted that the Examining Division has mischaracterised the Board's Decision. T657/10 states in paragraph 12.2 of the Reasons for the Decision that:

> "*As a common denominator to all cases underlying these decisions, the disclosure of the patent and/or of the patent application has to enable a skilled person to obtain the particular product resulting from the "elite event" without the need to repeat the random method de novo,* ***be it by*** *a deposit of said product (microorganism, monoclonal antibody, etc.) or by* ***a disclosure of structural features or elements of said product (such as flanking sequences of the genome insertion site, specific mutated nucleic acid sequence, etc.)****. In other words, the disclosure of the patent or patent application is sufficient for a skilled person if he/she can obtain the particular product without having to rely on pure chance again, i.e. on the screening and selection of random natural events. If this is the case the requirements of Article 83 EPC are considered to be fulfilled.*" (Emphasis added)

Thus, T657/10 essentially establishes that an event is sufficiently disclosed where structural features or elements of the product is disclosed. In contrast to the fact pattern underlying Decision T657/10, the present application provides structural features or elements of the product, i.e., 5' and 3' flanking sequences structurally defining a specific genomic insertion site.

Therefore, there is no reason to believe that the skilled person would not have been in a position to put the claimed invention into effect, and so the claimed invention must be sufficiently disclosed. Accordingly, the amended claims meet the requirements of Article 83 EPC.

Concluding Remarks

The Application is now believed to be in an allowable form and the Examiner is asked to reconsider it favourably. If, however, there are any queries outstanding, please issue a further Examination Report or telephone the undersigned. Only as a precaution in case the Examiner is minded to reject the Application without issuing first a further Examination Report, I request Oral Proceedings.

I look forward to hearing from the Examiner in due course.

Yours faithfully

Electronically Signed
A BENTHAM
ASSOCIATION NO. 811

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Amended claims (clean copy)	Amended claims (clean) N96314A-EP AXB KKT.PDF	CLMS-1.PDF
2	Amended claims with annotations	Amended claims (tracked) N96314A-EP AXB KKT.PDF	CLMS-HWA-1.PDF
3	Reply to examination report	Response to Examination Report N96314A-EP AXB KKT.PDF	EXRE3-1.PDF

	Payment	
1	Method of payment	Not specified

Signatures

Place:	**Oxford, GB**
Date:	**04 January 2019**
Signed by:	**Kapil Tuladhar 55592**
Association:	**J A Kemp**
Representative name:	**Kapil TULADHAR**
Capacity:	**(Representative)**

CLAIMS

1. An insect resistant TC1507 corn plant or parts thereof characterised by SEQ ID NO: 24, or TC1507 corn seed characterised by SEQ ID NO: 24.

2. The plant or seed of claim 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

CLAIMS

1. An insect resistant TC1507 corn plant or parts thereof characterised by SEQ ID NO: 24, or TC1507 corn seed characterised by SEQ ID NO: 24~~, comprising the nucleotide sequence of SEQ ID NO: 24~~.

2. The plant or seed of claim 1, obtainable by:

(a) sexually crossing a first insect resistant parental corn line comprising SEQ ID NO: 24, and a second parental corn line that lacks insect resistance, to produce a first plurality of progeny plants; and

(b) selecting from the first plurality of progeny plants of (a) a progeny plant that is insect resistant; and

(c) optionally selfing the insect resistant progeny plant of (b) to produce a second plurality of progeny plants, and selecting from the second plurality of progeny plants an insect resistant plant; and

(d) optionally back-crossing the insect resistant progeny plant of (b) or the insect resistant progeny plant of (c), to the second parental corn plant of (a) or a third parental corn plant.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	7061057
Application number	EP15168051.9
Date of receipt	04 January 2019
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) CLMS-HWA-1.PDF\Amended claims (tracked) N96314A-EP AXB KKT.PDF (1 p.) ep-sfd-request.xml CLMS-1.PDF\Amended claims (clean) N96314A-EP AXB KKT.PDF (1 p.) EXRE3-1.PDF\Response to Examination Report N96314A-EP AXB KKT.PDF (3 p.)
Submitted by	CN=Kapil Tuladhar 55592
Method of submission	Online
Date and time receipt generated	04 January 2019, 17:40 (CET)
Message Digest	76:33:34:61:6E:F8:4C:2C:D0:DD:EC:B9:0D:55:D4:E1:73:E3:7A:A3

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Wezyk, Magdalena

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
27.02.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402

Applicant/Proprietor
Dow AgroSciences, LLC, et al

Summons to attend oral proceedings pursuant to Rule 115(1) EPC

You are hereby summoned to attend oral proceedings arranged in connection with the above-mentioned European patent application.

The matters to be discussed are set out in the communication accompanying this summons (EPO Form 2906).

The oral proceedings, which will not be public, will take place before the Examining Division

on 13.09.19 at 09.00 hrs at the EPO,
PschorrHöfe, Bayerstr. 34, 80335 Munich

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 2009, 68). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC, see also OJ EPO 2008, 471).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to Special edition No. 3 OJ EPO 2007, L.1, concerning the filing of authorisations for company employees and lawyers acting as representatives before the EPO.

The final date for making written submissions and/or amendments (R. 116 EPC) is 13.08.19.

The actual room number as well as the waiting room numbers will be given to you by the porter in the foyer at the above EPO address.

Parking is available in the underground car park, accessible only via the entrance "Grasserstrasse 2/6". On presentation of the summons to oral proceedings at one of the porters' lodges in the PschorrHöfe, the parking ticket will be revoked.

Please note that the oral proceedings will always take place as scheduled unless you are otherwise informed. The EPO will generally not send a reminder or a further communication prior to the date of oral proceedings.

1st Examiner:	2nd Examiner:	Chairman:
Helliot, Bertrand	Rutz, Berthold	Krüger, Julia

Registered letter with advice of delivery
EPO Form 2008 09.18 [ORAL03=9999] (20/02/19)

to EPO postal service: 20.02.19

For the Examining Division

Annexes:
Confirmation of receipt (Form 2936)
Communication (EPO Form 2906)

The examination is being carried out on the **following application documents**

Description, Pages

1-39	filed in electronic form on	01-06-2018

Sequence listings, SEQ ID NO

1-57	as originally filed

Claims, Numbers

1, 2	filed in electronic form on	04-01-2019

Drawings, Sheets

1/1	as originally filed

1 INTRODUCTION

1.1 After having carefully considered the arguments provided by the Applicant in his reply of January, 4th 2019, the Examining Division is not convinced by said arguments and does still not consider the set of claims on file to comply with the requirements of the EPC.

1.2 Therefore, in order to expedite the procedure and in accordance with the Applicant's request, oral proceedings are arranged for the present file in order to arrive at a decision on the outstanding matter outlined below.

1.3 In case the applicant does not intend to participate at the Oral Proceedings, the applicant is informed of the possibility to request a decision according to the state of the file (Guidelines C-V, 15 and E-X, 2.5) open to appeal. The request should be submitted in written form and not contain any further amendments or arguments. At the same time the request for oral proceedings should be withdrawn. The applicant is to note that a declaration of

non-appearance at Oral Proceedings is equivalent to a withdrawal of the request for Oral Proceedings (T3/90). If the applicant files further amendments or arguments, Oral Proceedings may take place even in the absence of the applicant and a decision according to the state of the file cannot be issued.

2 SUPPORT (Art. 83 EPC)

It is clear from the description that the DNA molecule of 6.2Kb, designated as PHI8999A (see Fig. 1), was used to transform maize embryo tissue by particle bombardment and regeneration of transgenic plants containing the Cry1F gene. The TC1507 event was selected from a population of independent transgenic events based on superior combination of characteristics, including insect resistance and agronomic performance.

For assessing the patentability of living matter the issue of reproducibility is of the foremost importance. Reproducibility can be assured by two ways:

- either by a deposit of living matter (seeds, microbiological strains). In that case it is required that the deposited material is publicly available and that the invention can actually be reproduced from such deposited material. If for example a novel and inventive trait is due to a single transgene, a skilled person can reproduce the invention from a living sample. If instead the claimed trait is dependent on a large number of loci in the genome, these will segregate in subsequent generations and it will be an undue burden to reproduce the invention from the deposited sample;

- an invention comprising living matter is also reproducible if a gene sequence responsible for the claimed trait is given in the application as filed together with instructions on how to repeatably obtain such an altered sequence in a target organism (e.g. by CRISPR-Cas).

In the present case, the present application is silent as to a deposited material that would have rendered the TC1507 corn plant publicly available. The Applicant fails as well in his letter dated January, 4th 2019, to provide such deposit evidence.

Accordingly, it seems that the reproducibility of the TC1507 event could not be assured by a deposit of living matter.

Repeating the transformation of maize embryo tissue by particle bombardment and the selection of a transgenic plant based on the claimed features has to be considered to put undue burden on the skilled person (see T657/10). However, the Applicant seems to opine that the skilled person would have been in the position to put the claimed invention into effect. Thus, in his letter dated January, 4th 2019, the Applicant argues that the present application provides structural features or elements of the product, i.e. 5' and 3' flanking sequences structurally defining a specific genomic insertion site.

The Examining Division respectfully disagrees with the Applicant's contention. Thus, the claimed subject-matter includes a particular event resulting from a random method and having one surprising and advantageous property, i.e. insect resistance. However, genome editing technologies, such as the CRISPR-Cas technology, were not available to the public at the relevant date of the present application. Moreover, as the applicant fails to provide any alternative thereof, the Examining Division has serious doubt that the skilled person would have been in the position to reproduce the TC1507 event.

Accordingly, it seems that the reproducibility of the TC1507 event cannot be assured either by the skilled person at the relevant date of the application.

The Examining Division is therefore still of the opinion that the claimed subject-matter violates the requirements of Art. 83 EPC.

3 REMARK

Finally, in the case this objection would be overcome by filing evidences that the claimed subject-matter was indeed available to the public, the Applicant is informed that the claimed subject-matter would violate Art. 54 EPC.

4 CONCLUDING REMARK

Any information the Applicant wishes to file in preparation for or in order to avoid the oral proceedings should be submitted at least one month before the date of the oral proceedings (R. 116(1) EPC).

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Wezyk, Magdalena

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
27.02.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

EPA/EPO/OEB Formblatt/Form/Formulaire : 2008

Empfangsbescheinigung über den Zugang des vorstehend bezeichneten Schriftstücks
Acknowledgement of receipt of the document specified above
Récépissé du document spécifié ci-dessus

Unter Bezugnahme auf die Mitteilung im ABl EPA 7/2010, 377 wird gebeten, die Empfangsbescheinigung mit Empfangsdatum und Unterschrift zu versehen und **umgehend** an das EPA zurückzusenden:

With reference to the Notice in OJ EPO 7/2010, 377, you are requested to date and sign the acknowledgement of receipt and return it to the EPO **immediately**:

Conformément au communiqué paru au JO OEB 7/2010, 377, vous êtes prié d'indiquer sur le récépissé la date de réception du document, de signer le récépissé et de le renvoyer **sans délai** à l' OEB:

- **über die Online-Dienste des EPA** (als Anlage zu EPA Form 1038) / **through EPO Online Services** (as annex to EPO Form 1038) / **par les services en ligne de l'OEB** (en tant que pièce jointe au formulaire OEB 1038),
- **per Fax / by fax / par téléfax (+49 (0) 89 2399-4465 or +31 (0) 70 340-3016)**
- oder per Post / or by post / ou par courrier.

Empfangen am / Received on / Reçu le :

Unterschrift / Signature:

Empfangsberechtigter/authorised recipient/
le destinataire ou la personne dûment mandatée

Rücksende-Adresse / Return address / Adresse de retour
(Umschlag / envelope / enveloppe ISO C4 / DL / C6/C5 / C6)

Europäisches Patentamt
80298 MÜNCHEN
DEUTSCHLAND

Rücksende-Adresse / Return address / Adresse de retour

EPA/EPO/OEB Form 2936 08.10

MS50950

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Acknowledgement	EPO Acknowledgement N96314A-EP AXB KKT.PDF	ADVOFDELIVRY-1.PDF

	Payment	
1	Method of payment	Not specified

Signatures

Place:	**Oxford, GB**
Date:	**05 March 2019**
Signed by:	**Kapil Tuladhar 55592**
Association:	**J A Kemp**
Representative name:	**Kapil TULADHAR**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication
Contact Customer Services at www.epo.

Wezyk, Magdalena

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
27.02.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

EPA/EPO/OEB Formblatt/Form/Formulaire : 2008

Empfangsbescheinigung über den Zugang des vorstehend bezeichneten Schriftstücks
Acknowledgement of receipt of the document specified above
Récépissé du document spécifié ci-dessus

Unter Bezugnahme auf die Mitteilung im ABl EPA 7/2010, 377 wird gebeten, die Empfangsbescheinigung mit Empfangsdatum und Unterschrift zu versehen und **umgehend** an das EPA zurückzusenden:

With reference to the Notice in OJ EPO 7/2010, 377, you are requested to date and sign the acknowledgement of receipt and return it to the EPO **immediately**:

Conformément au communiqué paru au JO OEB 7/2010, 377, vous êtes prié d'indiquer sur le récépissé la date de réception du document, de signer le récépissé et de le renvoyer **sans délai** à l' OEB:

- **über die Online-Dienste des EPA** (als Anlage zu EPA Form 1038) / **through EPO Online Services** (as annex to EPO Form 1038) / **par les services en ligne de l'OEB** (en tant que pièce jointe au formulaire OEB 1038),
- **per Fax / by fax / par téléfax (+49 (0) 89 2399-4465 or +31 (0) 70 340-3016)**
- oder per Post / or by post / ou par courrier.

Empfangen am / Received on / Reçu le :

4 March 2019

Unterschrift / Signature:

Empfangsberechtigter/authorised recipient/
le destinataire ou la personne dûment mandatée

Rücksende-Adresse / Return address / Adresse de retour
(Umschlag / envelope / enveloppe ISO C4 / DL / C6/C5 / C6)

Europäisches Patentamt
80298 MÜNCHEN
DEUTSCHLAND

Rücksende-Adresse / Return address / Adresse de retour

EPA/EPO/OEB Form 2936 08.10

MS50950

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	7253715
Application number	EP15168051.9
Date of receipt	05 March 2019
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml ADVOFDELIVRY-1.PDF\EPO Acknowledgement N96314A-EP AXB KKT.PDF (1 p.)
Submitted by	CN=Kapil Tuladhar 55592
Method of submission	Online
Date and time receipt generated	05 March 2019, 17:41 (CET)
Message Digest	F2:82:49:D1:C4:96:9A:EF:D5:BF:4B:76:0D:8B:A8:81:D8:82:45:C2

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

J A • K E M P

BY ONLINE FILING

The European Patent Office
Bayerstrasse 34
(entrance via Zollstrasse 3)
80335 Munich
Germany

13 March 2019

Dear Sirs

European Patent Application No. 15168051.9
DOW AGROSCIENCES LLC *et al.*
Our Ref: N96314A-EP AXB/KKT/ncb

I refer to the summons dated 27 February 2019, appointing oral proceedings for Friday 13 September 2019. A change of date is requested.

The oral proceedings for the present application are scheduled for the day immediately after a three day oral proceedings already scheduled for patent no. 2787075 (application no. 14172420.3) on Tuesday 10 September to Thursday 12 September 2019 before the opposition division. Accordingly, the requirements set out in GL Section E-III 7.1.1 for change of date for the oral proceedings for the present application appear to be met.

The examining division will appreciate that the present application is a divisional of patent no. 1620571 (application no. 04751097.9), on which I am also the representative. Taken together, the parent and divisional have a complex history including some unusual background facts (including that the GM event around which this application is based is one of the very few to have received EU approval) and I have been working on them for well over ten years. I am also responsible for many other applications in the plant science area for two of the present three Applicants. For the purposes of continuity, is therefore desirable that I be able represent the Applicants at any oral proceedings appointed for the present application.

If a change of date is not possible, I should be available on 13 September as long as my previous oral proceedings finishes on time. However, as the three-day footprint suggests, that is a very large and complex case, which itself is a second-generation divisional whose parent/divisional have themselves been opposed and are currently in litigation in the UK. Previous experience in that portfolio suggests that it cannot

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • PARIS • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

be excluded that the oral proceedings will overrun or finish late enough even on a third day that I am unable to prepare properly for this hearing the following day.

As to alternative dates, I request that the following dates are also avoided:

- 11-13 June 2019 (oral proceedings on application no. 13813541.3);
- 28 June to 12 July 2019 inclusive (pre-booked non-refundable holiday in North America; details available if required);
- 15 and 16 July 2019 (and if possible that whole week), to allow time to prepare after holiday absence;
- 29-31 July 2019 (oral proceedings on application no. 13721544.8);
- 9-12 September 2019 (oral proceedings on application no. 14172420.3);
- 16-19 September 2019 (oral proceedings in The Hague on application no.14831132.7);
- 23 September 2019 (oral proceedings on application no.14761477.0);
- 24-27 September 2019 (oral proceedings on application no. 11162681.8);
- 21 and 22 November 2019 (already-rescheduled oral proceedings on application no. 12778014.6); and
- 2-5 December 2019 (already-rescheduled oral proceedings on application no.10184427.2); and
- 10-13 February 2020 (UK Supreme Court hearing in above-mentioned litigation).

I apologise for the complexity of the above but hope that these comments will help the examining division find a suitable date for oral proceedings for this application.

I look forward to hearing about this in due course. If the examining division has any questions, please let me know.

Yours faithfully

Electronically Signed
ANDREW BENTHAM
ASSOCIATION NO. 811

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Letter dealing with Oral proceedings	Letter Regarding Summons N96314A-EP AXB KKT.PDF	ORAL-1.PDF

	Payment	
1	Method of payment	Not specified

Signatures

Place:	
Date:	**13 March 2019**
Signed by:	**Andrew Bentham 23464**
Association:	**J A Kemp**
Representative name:	**Andrew BENTHAM**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	7280017
Application number	EP15168051.9
Date of receipt	13 March 2019
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml ORAL-1.PDF\Letter Regarding Summons N96314A-EP AXB KKT.PDF (2 p.)
Submitted by	CN=Andrew Bentham 23464
Method of submission	Online
Date and time receipt generated	13 March 2019, 16:59 (CET)
Message Digest	99:13:09:EB:C6:78:C1:25:A0:4F:60:CA:11:25:57:29:7F:55:D2:0E

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Costa, Gwendal

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date: 28.03.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402

Applicant/Proprietor: Dow AgroSciences, LLC, et al

BRIEF COMMUNICATION

Oral Proceedings on 13.09.19 at 09:00 hours

Subject:
- [x] Your letter of 13.03.19.....
- [ ] Maintenance / Cancellation / postponement at the instigation of the division
- [ ] Consultation by telephone on.....(EPO Form 2036)

Communication:

1. [ ] The date / time fixed for oral proceedings is maintained.
   - [ ] The reasons are indicated on enclosed EPO Form 2906.
2. [ ] The above-mentioned oral proceedings will start at ..... hours.
3. [x] The summons to attend oral proceedings on the date indicated above is cancelled.

3.1
- [ ] The reasons are indicated on enclosed EPO Form 2906.
- [ ] The procedure will be continued in writing.
- [x] A new date will be set later.
- [ ] New summons will follow.

3.2 [ ] For administrative reasons the oral proceedings are postponed to a later date. New summons will follow.

3.3 [ ] The application is deemed to be withdrawn. The right to oral proceedings only persists as long as proceedings are pending.

4. [ ] The request for the oral proceedings to be held by videoconference:

4.1 [ ] is allowed.

4.2 [ ] is rejected.
- [ ] No videoconference studio is available on the date indicated above.
- [ ] See enclosed EPO Form 2906.

Registered letter

5. ☐ Please confirm receipt of this communication by return fax with date stamp and signature to the EPO central fax server in Munich: (+49) (0) 89 23 99 44 65.

Please take note.

For the Examining Division

Enclosure(s): ☐ EPO Form 2906

☐ EPO Form 2036

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Costa, Gwendal

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

The former summons dated 27.02.2019 are superseded herewith

Date
01.04.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Summons to attend oral proceedings pursuant to Rule 115(1) EPC

You are hereby summoned to attend oral proceedings arranged in connection with the above-mentioned European patent application.

The matters to be discussed are set out in the communication accompanying this summons (EPO Form 2906).

The oral proceedings, which will not be public, will take place before the Examining Division

on 06.11.19 at 09.00 hrs at the EPO,
PschorrHöfe, Bayerstr. 34, 80335 Munich

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 2009, 68). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC, see also OJ EPO 2008, 471).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to Special edition No. 3 OJ EPO 2007, L.1, concerning the filing of authorisations for company employees and lawyers acting as representatives before the EPO.

The final date for making written submissions and/or amendments (R. 116 EPC) is 04.10.19.

The actual room number as well as the waiting room numbers will be given to you by the porter in the foyer at the above EPO address.

Parking is available in the underground car park, accessible only via the entrance "Grasserstrasse 2/6". On presentation of the summons to oral proceedings at one of the porters' lodges in the PschorrHöfe, the parking ticket will be revoked.

Please note that the oral proceedings will always take place as scheduled unless you are otherwise informed. The EPO will generally not send a reminder or a further communication prior to the date of oral proceedings.

1st Examiner:	2nd Examiner:	Chairman:
Helliot, Bertrand	Rutz, Berthold	Krüger, Julia

Registered letter with advice of delivery
EPO Form 2008 09.18 [ORAL03=9999] (25/03/19)

to EPO postal service: 25.03.19

For the Examining Division

Annexes:
Confirmation of receipt (Form 2936)
~~Communication (EPO Form 2906)~~

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Costa, Gwendal

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
01.04.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

EPA/EPO/OEB Formblatt/Form/Formulaire : 2088

Empfangsbescheinigung über den Zugang des vorstehend bezeichneten Schriftstücks
Acknowledgement of receipt of the document specified above
Récépissé du document spécifié ci-dessus

Unter Bezugnahme auf die Mitteilung im ABl EPA 7/2010, 377 wird gebeten, die Empfangsbescheinigung mit Empfangsdatum und Unterschrift zu versehen und **umgehend** an das EPA zurückzusenden:

With reference to the Notice in OJ EPO 7/2010, 377, you are requested to date and sign the acknowledgement of receipt and return it to the EPO **immediately**:

Conformément au communiqué paru au JO OEB 7/2010, 377, vous êtes prié d'indiquer sur le récépissé la date de réception du document, de signer le récépissé et de le renvoyer **sans délai** à l' OEB:

- **über die Online-Dienste des EPA** (als Anlage zu EPA Form 1038) / **through EPO Online Services** (as annex to EPO Form 1038) / **par les services en ligne de l'OEB** (en tant que pièce jointe au formulaire OEB 1038),
- **per Fax / by fax / par téléfax (+49 (0) 89 2399-4465 or +31 (0) 70 340-3016)**
- oder per Post / or by post / ou par courrier.

Empfangen am / Received on / Reçu le :

Unterschrift / Signature:

Empfangsberechtigter/authorised recipient/
le destinataire ou la personne dûment mandatée

Rücksende-Adresse / Return address / Adresse de retour
(Umschlag / envelope / enveloppe ISO C4 / DL / C6/C5 / C6)

Europäisches Patentamt
80298 MÜNCHEN
DEUTSCHLAND

Rücksende-Adresse / Return address / Adresse de retour

EPA/EPO/OEB Form 2936 08.10

MS50950

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Acknowledgement	Signed Acknowledgement N96314A-EP.PDF	ADVOFDELIVRY-1.PDF

	Payment	
1	Method of payment	Not specified

Signatures

Place:	**Oxford, GB**
Date:	**09 April 2019**
Signed by:	**Kapil Tuladhar 55592**
Association:	**J A Kemp**
Representative name:	**Kapil TULADHAR**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Costa, Gwendal

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
01.04.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

EPA/EPO/OEB Formblatt/Form/Formulaire : 2088

Empfangsbescheinigung über den Zugang des vorstehend bezeichneten Schriftstücks
Acknowledgement of receipt of the document specified above
Récépissé du document spécifié ci-dessus

Unter Bezugnahme auf die Mitteilung im ABl EPA 7/2010, 377 wird gebeten, die Empfangsbescheinigung mit Empfangsdatum und Unterschrift zu versehen und **umgehend** an das EPA zurückzusenden:

With reference to the Notice in OJ EPO 7/2010, 377, you are requested to date and sign the acknowledgement of receipt and return it to the EPO **immediately**:

Conformément au communiqué paru au JO OEB 7/2010, 377, vous êtes prié d'indiquer sur le récépissé la date de réception du document, de signer le récépissé et de le renvoyer **sans délai** à l' OEB:

- **über die Online-Dienste des EPA** (als Anlage zu EPA Form 1038) / **through EPO Online Services** (as annex to EPO Form 1038) / **par les services en ligne de l'OEB** (en tant que pièce jointe au formulaire OEB 1038),
- **per Fax / by fax / par téléfax (+49 (0) 89 2399-4465 or +31 (0) 70 340-3016)**
- oder per Post / or by post / ou par courrier.

Empfangen am / Received on / Reçu le :

8 April 2019

Unterschrift / Signature:

Empfangsberechtigter/authorised recipient/
le destinataire ou la personne dûment mandatée

Rücksende-Adresse / Return address / Adresse de retour
(Umschlag / envelope / enveloppe ISO C4 / DL / C6/C5 / C6)

Europäisches Patentamt
80298 MÜNCHEN
DEUTSCHLAND

Rücksende-Adresse / Return address / Adresse de retour

EPA/EPO/OEB Form 2936 08.10

MS50950

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	7367543
Application number	EP15168051.9
Date of receipt	09 April 2019
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml ADVOFDELIVRY-1.PDF\Signed Acknowledgement N96314A-EP.PDF (1 p.)
Submitted by	CN=Kapil Tuladhar 55592
Method of submission	Online
Date and time receipt generated	09 April 2019, 15:41 (CEST)
Message Digest	E6:CE:A0:62:4F:F0:72:2A:78:7F:24:24:BA:0E:8E:45:03:35:D5:EE

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

J A · K E M P

BY ONLINE FILING

The European Patent Office
80298 Munich
Germany

6 September 2019

Dear Sirs

European Patent Application No. 15168051.9
DOW AGROSCIENCES LLC *et al*.
Our Ref: N96314A-EP AXB/KKT

I refer to the outstanding Summons to attend oral proceedings on 6 November 2019.

As the examining division will appreciate, at least pending claim 2 relates to a plant obtainable by an essentially biological process (see step (a) of claim 2). In light of Rule 28(2) EPC and the questions over its legitimacy under Article 164(2) EPC (c.f. T1063/18), the patentability of this subject matter under Article 53(b) EPC is of course currently under consideration by the Enlarged Board of Appeal in the referral made by the EPO president and pending as case G3/19.

With reference to the EPO's Notice of 9 April 2019 (OJ EPO 2019 A34), we therefore request that the oral proceedings are cancelled and that examination proceedings are stayed until case G3/19 has been decided.

We look forward to hearing from the examining division.

Yours faithfully

Electronically Signed
A BENTHAM
ASSOCIATION NO. 811

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • PARIS • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**

A list of our partners is available at our principal place of business at the address above. Regulated by IPREG

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Letter dealing with oral proceedings during the appeal procedure	Response to Summons to Oral Proceedings N96314A-EP AXB KKT.PDF	APPEAL-ORAL.PDF

	Payment	
1	Method of payment	Not specified

Signatures

Place:	**Oxford, GB**
Date:	**06 September 2019**
Signed by:	**Kapil Tuladhar 55592**
Association:	**J A Kemp**
Representative name:	**Kapil TULADHAR**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	7834504
Application number	EP15168051.9
Date of receipt	06 September 2019
Receiving Office	European Patent Office, The Hague
Your reference	N96314A-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml APPEAL-ORAL.PDF\Response to Summons to Oral Proceedings N96314A-EP AXB KKT.PDF (1 p.)
Submitted by	CN=Kapil Tuladhar 55592
Method of submission	Online
Date and time receipt generated	06 September 2019, 13:59 (CEST)
Message Digest	8D:E7:E1:89:FB:37:99:23:B5:09:79:02:F2:74:6C:76:75:AC:65:E3

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

Application No. : 15 168 051.9

Consultation by telephone with the applicant / representative

Despatch for information

Participants

Applicant: Dow AgroSciences, LLC

Representative: Cullem, Nathalie

Member(s) of the Examining Division: Helliot, Bertrand

Result of consultation

See Separate Sheet

17.09.2019
. . . . . .
Date

Helliot, Bertrand
. . . . . .
Examiner

Enclosure(s):

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME-UNI

Formalities Officer
Name: Büchler, Stefanie
Tel: +49 89 2399 - 8090
or call
+31 (0)70 340 45 00

Substantive Examiner
Name: Helliot, Bertrand
Tel: +49 89 2399 - 7793

Application No.	Ref.	Date
15 168 051.9 - 1118	N96314A-EP	18.09.2019
Applicant: Dow AgroSciences, LLC, et al		

Result of consultation

A copy of the result of consultation of 17.09.2019 is enclosed for your information.

Europäisches Patentamt • European Patent Office • Office européen des brevets

Helliot, Bertrand
For the Examining Division

Enclosure(s): Copy of result of consultation (Form 2036)

The Applicant (Ms Cullem) was informed that it is not possible to stay the present proceedings as requested by the Applicant with letter September, 6th 2019, until the Enlarged Board of Appeal issues its decision (G 3/19) because the claimed subject-matter relates to transgenic plants. However, transgenic descendants are technically characterised by the transgene they carry. The technical effect of these transgenic plants or animals is the result of a technical process (the transformation step), and this technical step is crucial to obtain the product. Transgenic plants or animals cannot be obtained **exclusively** by means of an essentially biological process. As a consequence, transgenic plants or animals are patentable, regardless of their generation.

The Applicant is informed that oral proceedings are maintained as scheduled.

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Büchler, Stefanie

J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
20.09.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

BRIEF COMMUNICATION

Oral Proceedings on 06.11.19 at 09:00 hours

Subject:
- ☒ Your letter of 06.09.2019
- ☒ Maintenance / Cancellation / postponement at the instigation of the division
- ☐ Consultation by telephone on.....(EPO Form 2036)

Communication:

1. ☒ The date / time fixed for oral proceedings is maintained.
   - ☐ The reasons are indicated on enclosed EPO Form 2906.
2. ☐ The above-mentioned oral proceedings will start at ..... hours.
3. ☐ The summons to attend oral proceedings on the date indicated above is cancelled.
   - 3.1
     - ☐ The reasons are indicated on enclosed EPO Form 2906.
     - ☐ The procedure will be continued in writing.
     - ☐ A new date will be set later.
     - ☐ New summons will follow.
   - 3.2 ☐ For administrative reasons the oral proceedings are postponed to a later date. New summons will follow.
   - 3.3 ☐ The application is deemed to be withdrawn. The right to oral proceedings only persists as long as proceedings are pending.
4. ☐ The request for the oral proceedings to be held by videoconference:
   - 4.1 ☐ is allowed.
   - 4.2 ☐ is rejected.
     - ☐ No videoconference studio is available on the date indicated above.
     - ☐ See enclosed EPO Form 2906.

Registered letter

5. ☐ Please confirm receipt of this communication by return fax with date stamp and signature to the EPO central fax server in Munich: (+49) (0) 89 23 99 44 65.

Please take note.

For the Examining Division

Enclosure(s): ☐ EPO Form 2906

☐ EPO Form 2036

J A • K E M P

BY ONLINE FILING

The European Patent Office
80298 Munich
Germany

2 October 2019

Dear Sirs

European Patent Application No. 15168051.9
DOW AGROSCIENCES LLC *et al*.
Our Ref: N96314A-EP AXB/KKT

On behalf of the Applicants, I hereby withdraw this application.

Yours faithfully

Electronically Signed
A BENTHAM
FOR J A KEMP LLP
ASSOCIATION NO. 811

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • CAMBRIDGE • PARIS • MUNICH

14 South Square, Gray's Inn, London WC1R 5JJ
T +44 20 3077 8600 **F** +44 20 7430 1000
mail@jakemp.com **www.jakemp.com**

J A Kemp LLP is a Limited Liability Partnership, registered in England and Wales (registered number OC427581) and regulated by IPReg.
Our registered office is at 14 South Square, Gray's Inn, London WC1R 5JJ, UK. Our use of the word 'partner' refers to a member of J A Kemp LLP.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

J A Kemp LLP
811
14 South Square
Gray's Inn
London Greater London WC1R 5JJ
United Kingdom

Phone: +44 20 3077 8600
Fax: +44 20 7430 1000
E-mail: mail@jakemp.com

80298 Munich
Germany
Tel. +49(0)89 2399-0 | Fax -4465

P.O. Box 5818
2280 HV Rijswijk
Netherlands
Tel. +31(0)70 340-2040 | Fax -3016

10958 Berlin
Germany
Tel. +49(0)30 25901-0 | Fax -840

The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	15168051.9
Applicant's or representative's reference	N96314A-EP

	Description of document	Original file name	Assigned file name
1	Withdrawal of an application	Withdraw Application N96314A-EP AXB KKT.PDF	WDRA-1.PDF

	Payment	
1	Method of payment	Not specified

Signatures

Place:	
Date:	**02 October 2019**
Signed by:	**Andrew Bentham 23464**
Association:	**J A Kemp LLP**
Representative name:	**Andrew BENTHAM**
Capacity:	**(Representative)**

Europäisches Patentamt
European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	7919534	
Application number	EP15168051.9	
Date of receipt	02 October 2019	
Receiving Office	European Patent Office, The Hague	
Your reference	N96314A-EP	
Applicant	All applicants as on file	
Documents submitted	package-data.xml epf1038.pdf (1 p.)	ep-sfd-request.xml WDRA-1.PDF\Withdraw Application N96314A-EP AXB KKT.PDF (1 p.)
Submitted by	CN=Andrew Bentham 23464	
Method of submission	Online	
Date and time receipt generated	02 October 2019, 20:21 (CEST)	
Message Digest	52:A7:E2:69:88:EE:AC:70:B7:53:DB:61:52:9A:89:D5:BA:E4:95:A4	

Correction by the EPO of errors in debit instructions filed by eOLF
Errors in debit instructions filed by eOLF that are caused by the editing of Form 1038E entries or the continued use of outdated software (all forms) may be corrected automatically by the EPO, leaving the payment date unchanged (see decision T 152/82, OJ EPO 1984, 301 and point 6.3 ff ADA, Supplement to OJ EPO 10/2007).

/European Patent Office/

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Wezyk, Magdalena

J A Kemp LLP
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
14.10.2019

Reference	Application No./Patent No.
N96314A-EP	15168051.9 - 1118 / 2942402
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Acknowledgement of withdrawal of the European patent application

Receipt of the declaration of withdrawal of the European patent application on 02.10.19 is acknowledged.

The proceedings are terminated as from withdrawal of the application. Any fees that have been paid but have not fallen due will be refunded.

☐ The examination fee will be refunded at a rate of 100% (Art. 11(a) RFees and OJ EPO 2016, A49).

☐ The examination fee will be refunded at a rate of 50% (Art. 11(b) RFees and OJ EPO 2016, A49).

☒ The examination fee will not be refunded (Art. 11(b) RFees and OJ EPO 2016, A49), because the application is withdrawn after:

- ☒ expiry of the time limit set in the first communication under Art. 94(3) EPC.
- ☐ the date specified in accordance with Rule 116 EPC in the first summons to attend oral proceedings accompanied by a communication from the Examining Division complying with the requirements of Art. 94(3) EPC and R. 71(1) EPC (EPO Form 2008EC).
- ☐ the date of dispatch of the communication under Rule 71(3) EPC.

☒ The summons (EPO Form 2008/2008EC) dated 01.04.2019 to attend oral proceedings on 06.11.2019 is cancelled.

For the Examining Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

Registered letter
EPO Form 2077N 12.17 (04/10/19)