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

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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.

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 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 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.

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

(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 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.

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.

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.

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.

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.

PCT/US04/35538

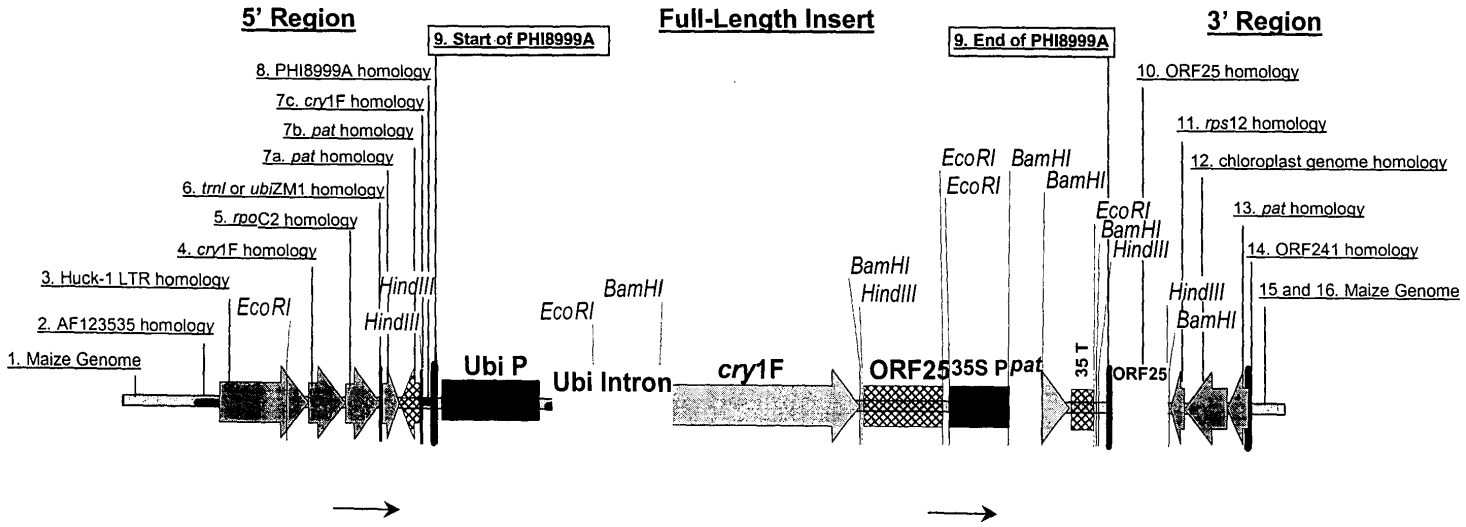


FIG. 1

SEQUENCE LISTING

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E. I. du Pont de Nemours and Company
Pioneer Hi-Bred International, Inc.

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Detection Thereof

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<223> Event specific primer sequence designed for TC1507

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<223> The sequence represents the transgenic insert in

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

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<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.

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cgtccgtttt cgtatttctc gggaaactct ggtatattcc cgtatttgtc ccgtattttc 300
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<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
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<210> 28
<211> 669
<212> DNA
<213> Zea mays

<400> 28
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gccagaccg 669

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

<400> 29
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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
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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
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>
<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
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 ccccccccccc 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
<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 gggagccccc 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
<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
tctcaaggcc 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

<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

<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
aaaccgcgga acacaagaac 20

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PCT/US04/13538

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☐ the International Searching Authority only
☐ the International Preliminary Examining Authority only

in connection with the international application identified below:

Title of the invention: **CORN EVENT TC1507 AND METHODS FOR DETECTION**

Applicant's or agent's file reference: **1523-PCT**

International application number (if already available): PCT/US04/13538

filed with the following Office: U.S. Patent Office as receiving Office and to make or receive payments on behalf of the undersigned.

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PCT/US04/13528 03092004

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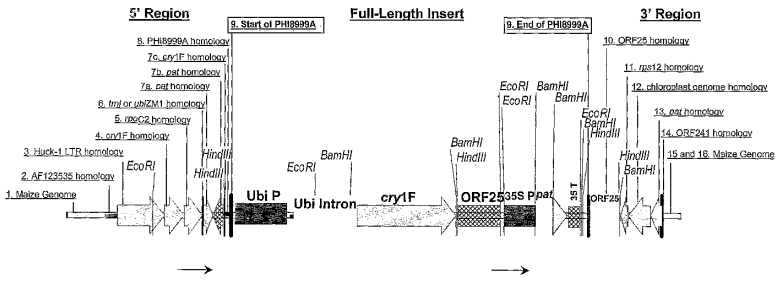
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[Continued on next page]

(54) Title: CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF



(57) Abstract: 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

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GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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.

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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 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 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.

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

(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 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.

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.

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.

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.

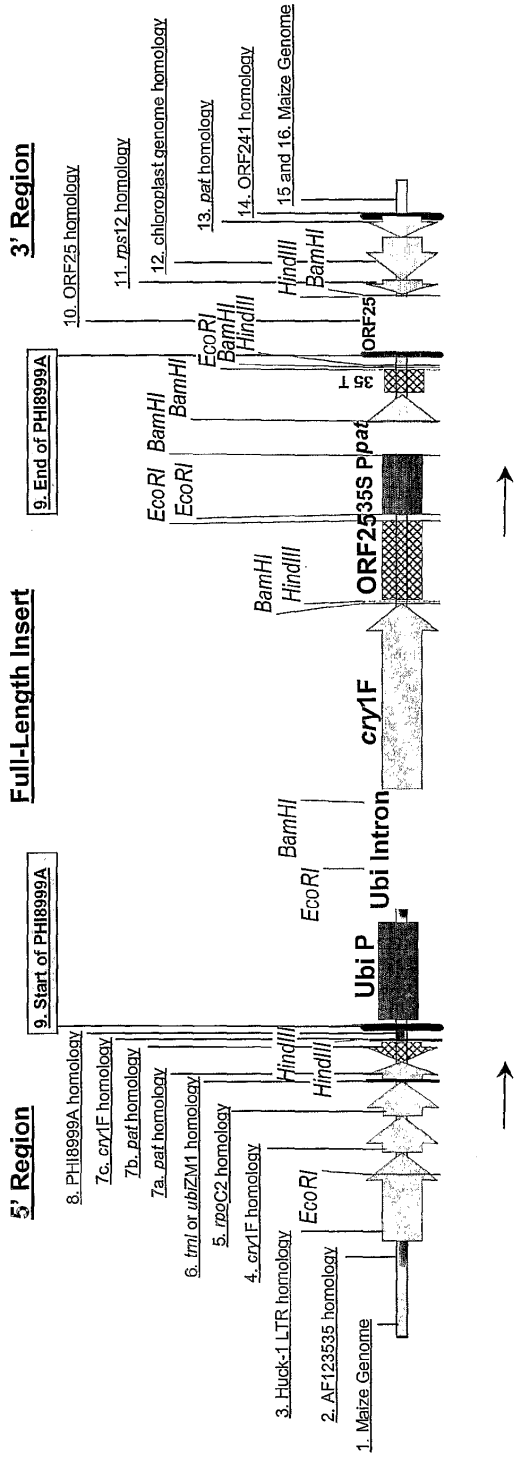

5' Region
Full-Length Insert
3' Region
1. Maize Genome
2. AF123535 homology
3. Huck-1 LTR homology
4. cry1F homology
5. rpoC2 homology
6. tml or ubiZM1 homology
7a. pat homology
7b. pat homology
7c. cry1F homology
8. PHI8999A homology
9. Start of PHI8999A
Ubi P
Ubi Intron
cry1F
ORF25
35S P
pat
35 t
9.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

FIG. 1

SEQUENCE LISTING

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E. I. du Pont de Nemours and Company
Pioneer Hi-Bred International, Inc.

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Detection Thereof

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

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

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<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.

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<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.

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

<400> 28
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gccagaccg 669

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

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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
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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
cggaagacga cgaactacgc ttcgcccgac cccagggctt ggactcagcc tcggctccgg 600
aagacgacga attccgcctc gcccgacccc agggctcgga ctcggcctcg gctccagaag 660
acgacgaact ccgcctcgcc cgaccccagg gctcggactc agcctcggct ccggaagacg 720
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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>
<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
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 ccccccccccc 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
<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
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agcccacgtc cgctcgtggg cccgagcctt ctttgcgtcc ttcttctcct tcctcttctc 480
cgcggtgacc ctccgcgcag ctcggtccac cgcatcctcc gggactggtg gcagggaagg 540
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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
<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
tctcaaggcc 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

<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

<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
aaaccgcgga acacaagaac 20

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

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

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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.

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Tracy Westphal
Typed or printed name of person signing Certificate

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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)

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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.

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☐ 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
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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

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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.

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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

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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.

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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);

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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

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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

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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

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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.

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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

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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.

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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

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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

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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

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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.

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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.

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"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.

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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

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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

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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

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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

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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

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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

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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

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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
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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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

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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.

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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

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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

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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

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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.

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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

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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.

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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

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(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

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(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.

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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.

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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.

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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.

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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.

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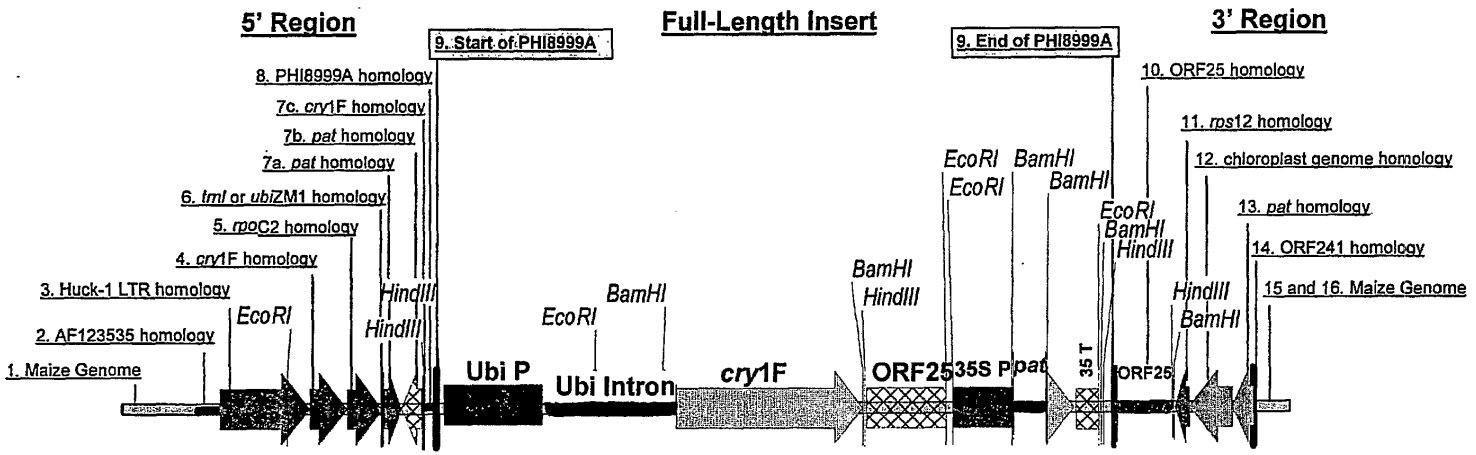


FIG. 1

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<210> 23
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<212> DNA

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<213> Artificial Sequence

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

<400> 23
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<210> 24
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<213> Artificial Sequence

<220>
<223> The sequence represents the transgenic insert in
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the insertion sites.

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

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<220>
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<210> 27
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<212> DNA
<213> Artificial Sequence

<220>
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<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

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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>

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<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

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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 ctttttcat 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

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<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

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<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

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<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

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<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

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

(57) Abstract: 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

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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.

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"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

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"&" 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 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	Authorized officer Helliot, B

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

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)

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Generaldirektion 1	Directorate General 1	Direction générale 1

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Date
22.09.05

Reference	Application No./Patent No.
	04751097.9 - 2405 PCT/US2004013538

Applicant/Proprietor
Dow Agrosciences LLC, et al

Entry into the European phase before the European Patent Office

These notes describe the procedural steps required for entry into the European phase before the European Patent Office (EPO). You are advised to read them carefully: failure to take the necessary action in time can lead to your application being deemed withdrawn.

1. The above-mentioned international patent application has been given European application No. **04751097.9**.

2. Applicants **without** a residence or their principal place of business in an EPC contracting state may themselves initiate European processing of their international applications, provided they do so before expiry of the 31st month from the priority date (see also point 6 below).

   During the European phase before the EPO as designated or elected Office, however, such applicants must be represented by a professional representative (Arts. 133(2) and 134(1), (7) EPC).

   Procedural acts performed after expiry of the 31st month by a professional representative who acted during the international phase but is not authorised to act before the EPO have no legal effect and therefore lead to loss of rights.

   Please note that a professional representative authorised to act before the EPO and who acted for the applicant during the international phase does not automatically become the representative for the European phase. Applicants are therefore strongly advised to appoint in good time any representative they wish to initiate the European phase for them; otherwise, the EPO has to send all communications direct to the applicant.

3. Applicants **with** a residence or their principal place of business in an EPC contracting state are not obliged to appoint, for the European phase before the EPO as designated or elected Office, a professional representative authorised to act before the EPO.
   However, in view of the complexity of the procedure it is recommended that they do so.

4. Applicants and professional representatives are also strongly advised to initiate the European phase using EPO Form 1200 (available free of charge from the EPO). This however is not compulsory.

5. **To enter the European phase before the EPO**, the following acts must be performed.
(N.B.: Failure validly to do so will entail loss of rights or other adverse legal consequences.)

5.1 If the EPO is acting as **designated** or **elected** Office (Arts. 22(1)(3) and 39(1) PCT respectively), applicants must, within 31 months from the date of filing or (where applicable) the earliest priority date:

a) Supply a translation of the international application into an EPO official language, if the International Bureau did not publish the application in such a language (Art. 22(1) PCT and Rule 107(1)(a) EPC).
If the translation is not filed in time, the international application is deemed withdrawn before the EPO (Rule 108(1) EPC).
This loss of rights is deemed not to have occurred if the translation is then filed within a two-month grace period as from notification of an EPO communication, provided a surcharge is paid at the same time (Rule 108(3) EPC).

b) Pay the national basic fee (EUR 160,00) and, where a supplementary European search report has to be drawn up, the search fee (EUR 960,00 ; Rule 107(1)(c) and (e) EPC).

c) If the time limit under Article 79(2) EPC expires before the 31-month time limit, pay the designation fee (EUR 75,00) for each contracting state designated (Rule 107(1)(d) EPC).

d) If the time limit under Article 94(2) EPC expires before the 31-month time limit, file the written request for examination **and** pay the examination fee (EUR 1430,00 ; Rule 107(1)(f) EPC).

e) Pay the third-year renewal fee (EUR 380,00) if it falls due before expiry of the 31-month time limit (Rule 107(1)(g) EPC).

If the fees under (b) to (d) above are not paid in time, or the written request for examination is not filed in time, the international application is deemed withdrawn before the EPO, or the contracting-state designation(s) in question is (are) deemed withdrawn (Rule 108(1) and (2) EPC). However, the fees may still be validly paid within a two-month grace period as from notification of an EPO communication, provided the necessary surcharges are paid at the same time (Rule 108(3) EPC). For the renewal fee under (e) above, the grace period is **six** months from the fee's due date (Article 86(2) EPC).

5.2 If the application documents on which the European grant procedure is to be based comprise more then ten claims, a claims fee is payable within the 31-month time limit under Rule 107(1) EPC for the eleventh and each subsequent claim (Rule 110(1) EPC). The fee can however still be paid within a one-month grace period as from notification of an EPO communication pointing out the failure to pay (Rule 110(2) EPC).

6. If the applicant had a representative during the application's international phase, the present notes will be sent to the representative, asking him to inform the applicant accordingly.

All subsequent communications will be sent to the applicant, or - if the EPO is informed of his appointment in time - to the applicant's European representative.

7. For more details about time limits and procedural acts before the EPO as designated and elected Office, see the EPO brochure

   How to get a European patent
   Guide for applicants - Part 2
   PCT procedure before the EPO - "Euro-PCT"

   This brochure, the list of professional representatives before the EPO, Form 1200 and details of the latest fees are now all available on the Internet under

   http://www.european-patent-office.org

RECEIVING SECTION

An das Europäische Patentamt | **To the European Patent Office** | **A l'Office européen des brevets** 1

EPO - Munich 85

07. Nov. 2005

Eintritt in die europäische Phase (EPA als Bestimmungsamt oder ausgewähltes Amt)

Entry into the European phase (EPO as designated or elected Office)

Entrée dans la phase européenne (l'OEB agissant en qualité d'office désigné ou élu)

Europäische Anmeldenummer oder, falls nicht bekannt, PCT-Aktenzeichen oder PCT-Veröffentlichungsnummer	European application number, or, if not known, PCT application or publication number	Numéro de dépôt de la demande de brevet européen ou, à défaut, numéro de dépôt PCT ou de publication PCT
	04751097.9 (PCT/US04/13538)	
Zeichen des Anmelders oder Vertreters (max. 15 Positionen)	Applicant's or representative's reference (max. 15 spaces)	Référence du demandeur ou du mandataire (15 caractères ou espaces au maximum)
	N.96314 AB	

☒ **1. Anmelder**
Die Angaben über den (die) Anmelder sind in der internationalen Veröffentlichung enthalten oder vom Internationalen Büro nach der internationalen Veröffentlichung vermerkt worden.

1. Applicant
Indications concerning the applicant(s) are contained in the international publication or recorded by the International Bureau after the international publication.

1. Demandeur
Les indications concernant le(s) demandeur(s) figurent dans la publication internationale ou ont été enregistrées par le Bureau international après la publication internationale.

☐ Änderungen, die das Internationale Büro noch nicht vermerkt hat, sind auf einem Zusatzblatt angegeben.

Changes which have not yet been recorded by the International Bureau are set out on an additional sheet.

Les changements qui n'ont pas encore été enregistrés par le Bureau international sont indiqués sur une feuille additionnelle.

Zustellanschrift
(siehe Merkblatt II, 1)

Address for correspondence
(see Notes II, 1)

Adresse pour la correspondance
(voir notice II, 1)

2. Vertreter

Name (Nur einen Vertreter angeben, der in das europäische Patentregister eingetragen und an den zugestellt wird)

Geschäftsanschrift

Telefon

Telefax **Telex**

☐ Weitere(r) Vertreter auf Zusatzblatt

2. Representative

Name (Name **only one** representative who will be listed in the Register of European Patents and to whom notification will be made)

BENTHAM, Andrew

Address of place of business

J.A. KEMP & CO.
14 South Square Gray's Inn
London WC1R 5JJ GB

Telephone

+44 20 7405 3292

Fax **Telex**

+44 20 7242 8932 23676

Additional representative(s) on additional sheet

2. Mandataire

Nom (N'indiquer **qu' un seul** mandataire, qui sera inscrit au Registre européen des brevets et auquel signification sera faite)

Adresse professionnelle

Zur Kasse

Téléphone

Téléfax **Télex**

Autre(s) mandataire(s) sur une feuille additionnelle

3. Vollmacht

☐ Einzelvollmacht ist beigefügt.

☐ Allgemeine Vollmacht ist registriert unter Nummer:

☐ Allgemeine Vollmacht ist eingereicht, aber noch nicht registriert.

☐ Die beim EPA als PCT-Anmeldeamt eingereichte Vollmacht schließt ausdrücklich die europäische Phase ein.

3. Authorisation

Individual authorisation is attached.

General authorisation has been registered under No:

A general authorisation has been filed, but not yet registered.

The authorisation filed with the EPO as PCT receiving Office expressly includes the European phase.

3. Pouvoir

Un pouvoir spécial est joint.

Un pouvoir général a été enregistré sous le n° :

Un pouvoir général a été déposé, mais n'est pas encore enregistré.

Le pouvoir général déposé à l'OEB agissant en qualité d'office récepteur au titre du PCT s'applique expressément à la phase européenne.

☒ **4. Prüfungsantrag**
Hiermit wird die Prüfung der Anmeldung gemäß Art. 94 EPÜ beantragt. Die Prüfungsgebühr wird (wurde) entrichtet.

Prüfungsantrag in einer zugelassenen Nichtamtssprache
(siehe Merkblatt III, 5.2) :

4. Request for examination
Examination of the application under Art. 94 EPC is hereby requested. The examination fee is being (has been, will be) paid.

Request for examination in an admissible non-EPO language
(see Notes III, 5.2) :

4. Requête en examen
Il est demandé que soit examinée la demande de brevet conformément à l'art. 94 CBE. Il est (a été, sera) procédé au paiement de la taxe d'examen.

Requête en examen dans une langue non officielle autorisée
(voir notice III, 5.2) :

☐ **5. Abschriften**
Zusätzliche Abschrift(en) der im ergänzenden europäischen Recherchenbericht angeführten Schriftstücke wird (werden) beantragt.

Anzahl der **zusätzlichen** Sätze von Abschriften

5. Copies
Additional copy (copies) of the documents cited in the supplementary European search report is (are) requested.

Number of **additional** sets of copies

5. Copies
Prière de fournir une ou plusieurs copies supplémentaires des documents cités dans le rapport complémentaire de recherche européenne.

Nombre de jeux **supplémentaires** de copies

6. Für das Verfahren vor dem EPA bestimmte Unterlagen

6.1 Dem Verfahren vor dem EPA als **Bestimmungsamt** (PCT I) sind folgende Unterlagen zugrunde zu legen:

☒ die vom Internationalen Büro **veröffentlichten Anmeldungsunterlagen** (mit **allen** Ansprüchen, Beschreibung und Zeichnungen), gegebenenfalls mit den geänderten Ansprüchen nach Art. 19 PCT

☒ soweit sie nicht ersetzt werden durch die beigefügten **Änderungen.**

Falls nötig, sind Klarstellungen auf einem Zusatzblatt einzureichen!

6.2 Dem Verfahren vor dem EPA als **ausgewähltem Amt** (PCT II) sind folgende Unterlagen zugrunde zu legen:

☒ die dem **internationalen vorläufigen Prüfungsbericht zugrunde gelegten Unterlagen,** einschließlich seiner eventuellen **Anlagen**
(Solche Anlagen müssen immer beigefügt werden)

☐ soweit sie nicht ersetzt werden durch die beigefügten **Änderungen.**

Falls nötig, sind Klarstellungen auf einem Zusatzblatt einzureichen!

☒ Sind dem EPA als mit der internationalen vorläufigen Prüfung beauftragten Behörde **Versuchsberichte** zugegangen, dürfen diese dem Verfahren vor dem EPA zugrunde gelegt werden.

6. Documents intended for proceedings before the EPO

6.1 Proceedings before the EPO as **designated Office** (PCT I) are to be based on the following documents:

the **application documents published** by the International Bureau (with **all** claims, description and drawings), where applicable with amended claims under Art. 19 PCT

unless replaced by the **amendments** enclosed.

Where necessary, clarifications must be submitted on a separate sheet!

6.2 Proceedings before the EPO as **elected Office** (PCT II) are to be based on the following documents:

the **documents on which the international preliminary examination report is based**, including its possible **annexes**
(Such annexes must always be filed)

unless replaced by the **amendments** enclosed.

Where necessary, clarifications must be submitted on a separate sheet!

If the EPO as International Preliminary Examining Authority has received **test reports**, these may be used as the basis of proceedings before the EPO.

6. Pièces destinées à la procédure devant l'OEB

6.1 La procédure devant l'OEB agissant en qualité **d'office désigné** (PCT I) doit se fonder sur les pièces suivantes :

les **pièces de la demande publiée** par le Bureau international (avec **toutes** les revendications, la description et les dessins), éventuellement avec les revendications modifiées conformément à l'article 19 du PCT

dans la mesure où elles ne sont pas remplacées par les **modifications** jointes.

Le cas échéant, des explications doivent être jointes sur une feuille additionnelle!

6.2 La procédure devant l'OEB **agissant en qualité d'office élu** (PCT II) doit se fonder sur les pièces suivantes :

les **pièces sur lesquelles se fonde le rapport d'examen préliminaire international**, y compris ses **annexes** éventuelles
(De telles annexes sont toujours à joindre)

dans la mesure où elles ne sont pas remplacées par les **modifications** jointes.

Le cas échéant, des explications doivent être jointes sur une feuille additionnelle!

Si l'OEB, agissant en qualité d'administration chargée de l'examen préliminaire international, a reçu des **rapports d'essais**, ceux-ci peuvent constituer la base de la procédure devant l'OEB.

	7. Übersetzungen Beigefügt sind die nachfolgend angekreuzten Übersetzungen in einer der Amtssprachen des EPA (Deutsch, Englisch, Französisch):	**7. Translations** Translations in one of the official languages of the EPO (English, French, German) are enclosed as crossed below:	**7. Traductions** Vous trouverez, ci-joint, les traductions cochées ci-après dans l'une des langues officielles de l'OEB (allemand, anglais, français) :
	• *Im Verfahren vor dem EPA als* ***Bestimmungsamt oder ausgewähltem Amt*** *(PCT I + II):*	• *In proceedings before the EPO as* ***designated or elected Office*** *(PCT I + II):*	• *Dans la procédure devant l'OEB agissant en qualité d'* ***office désigné ou élu*** *(PCT I + II):*
☐	Übersetzung der **ursprünglich eingereichten internationalen Anmeldung** (Beschreibung, Ansprüche, etwaige Textbestandteile in den Zeichnungen), der veröffentlichten Zusammenfassung, und etwaiger Angaben über biologisches Material nach Regel 13bis.3 und 13bis.4 PCT	Translation of the **international application** (description, claims, any text in the drawings) **as originally filed**, of the abstract as published and of any indication under Rule 13bis.3 and 13bis.4 PCT regarding biological material	Traduction de la **demande internationale telle que déposée initialement** (description, revendications, textes figurant éventuellement dans les dessins), de l'abrégé publié, et de toutes indications visées aux règles 13bis.3 et 13bis.4 du PCT concernant le matériel biologique
☐	Übersetzung der **prioritätsbegründenden Anmeldung(en)**	Translation of **the priority application(s)**	Traduction de la (des) **demande(s) ouvrant le droit de priorité**
☐	Es wird hiermit erklärt, daß die internationale Anmeldung in ihrer ursprünglich eingereichten Fassung eine vollständige Übersetzung der früheren Anmeldung ist (Regel 38(5) EPÜ)	It is hereby declared that the international application as originally filed is a complete translation of the previous application (Rule 38(5) EPC)	Il est déclaré par la présente que la demande internationale telle que déposée initialement est une traduction intégrale de la demande antérieure (règle 38(5) CBE)
	• ***Zusätzlich*** *im Verfahren vor dem EPA als* ***Bestimmungsamt*** *(PCT I):*	• ***In addition****, in proceedings before the EPO as* ***designated Office*** *(PCT I):*	• ***De plus****, dans la procédure devant l'OEB agissant en qualité d'* ***office désigné*** *(PCT I) :*
☐	Übersetzung der nach Art. 19 PCT **geänderten Ansprüche** nebst Erklärung, falls diese dem Verfahren vor dem EPA zugrunde gelegt werden sollen (siehe Feld 6)	Translation of **amended claims** and any statement under Art. 19 PCT, if the claims as amended are to form the basis for the proceedings before the EPO (see Section 6)	Traduction des **revendications modifiées** et de la déclaration faite conformément à l'article 19 du PCT, si la procédure devant l'OEB doit être fondée sur les revendications modifiées (voir la rubrique 6)
	• ***Zusätzlich*** *im Verfahren vor dem EPA als* ***ausgewähltem Amt*** *(PCT II):*	• ***In addition****, in proceedings before the EPO as* ***elected Office*** *(PCT II):*	• ***De plus****, dans la procédure devant l'OEB agissant en qualité d'* ***office élu*** *(PCT II) :*
☐	Übersetzung der **Anlagen zum internationalen vorläufigen Prüfungsbericht**	Translation of any **annexes to the international preliminary examination report**	Traduction des **annexes du rapport d'examen préliminaire international**
☐	**8. Biologisches Material** Die Erfindung bezieht sich auf bzw. verwendet biologisches Material, das nach Regel 28 EPÜ hinterlegt worden ist.	**8. Biological material** The invention relates to and/or uses biological material deposited under Rule 28 EPC.	**8. Matière biologique** L'invention concerne et/ou utilise de la matière biologique, déposée conformément à la règle 28 CBE.
☐	Die **Angaben nach Regel 28(1)c) EPÜ** (falls noch nicht bekannt, die Hinterlegungsstelle und das (die) Bezugszeichen [Nummer, Symbole usw.] des Hinterlegers) sind in der internationalen Veröffentlichung oder in der gemäß Feld 7 eingereichten Übersetzung enthalten auf:	The **particulars referred to in Rule 28(1)(c) EPC** (if not yet known, the depository institution and the identification reference(s) [number, symbols etc.] of the depositor) are given in the international publication or in the translation submitted under Section 7 on:	Les **indications visées à la règle 28(1)c) CBE** (si non encore connues, l'autorité de dépôt et la (les) référence(s) d'identification [numéro ou symboles etc.] du déposant) figurent dans la publication internationale ou dans une traduction produite conformément à la rubrique 7 à la / aux:
	Seite(n) / Zeile(n)	page(s) / line(s)	page(s) / ligne(s)
	Die **Empfangsbescheinigung(en)** der Hinterlegungsstelle	The **receipt(s) of deposit** issued by the depositary institution	Le(s) **récépissé(s) de dépôt** délivré(s) par l'autorité de dépôt
☐	ist (sind) beigefügt	is (are) enclosed	est (sont) joint(s)
☐	wird (werden) nachgereicht	will be filed at a later date	sera (seront) produit(s) ultérieurement
☐	Verzicht auf die Verpflichtung des Antragstellers nach Regel 28(3) EPÜ auf gesondertem Schriftstück	Waiver of the right to an undertaking from the requester pursuant to Rule 28(3) EPC attached.	Renonciation, sur document distinct, à l'engagement du requérant au titre de la règle 28(3) CBE.

9. Nucleotid- und Aminosäuresequenzen

☒ Die nach Regeln 5.2 und 13ter PCT sowie Regel 111(3) EPÜ erforderlichen Unterlagen liegen dem EPA bereits vor.

☐ Das schriftliche Sequenzprotokoll wird anliegend nachgereicht.

☐ Das Sequenzprotokoll geht nicht über den Inhalt der Anmeldung in der ursprünglich eingereichten Fassung hinaus.

☐ Der vorgeschriebene Datenträger ist beigefügt.

☐ Die auf dem Datenträger gespeicherte Information stimmt mit dem schriftlichen Sequenzprotokoll überein.

9. Nucleotide and amino acid sequences

The items necessary in accordance with Rules 5.2 and 13ter PCT and Rule 111(3) EPC have already been furnished to the EPO.

The written sequence listing is furnished herewith.

The sequence listing does not include matter which goes beyond the content of the application as filed.

The prescribed data carrier is enclosed.

The information recorded on the data carrier is identical to the written sequence listing.

9. Séquences de nucléotides et d'acides aminés

Les pièces requises selon les règles 5.2 et 13ter PCT et la règle 111(3) CBE ont déjà été déposées auprès de l'OEB.

La liste de séquences écrite est produite ci-joint.

La liste de séquences ne contient pas d'éléments s'étendant au-delà du contenu de la demande telle qu'elle a été déposée.

Le support de données prescrit est joint.

L'information figurant sur le support de données est identique à celle que contient la liste de séquences écrite.

10. Benennungsgebühren

☒ 10.1 Es ist derzeit beabsichtigt, den **siebenfachen** Betrag einer Benennungsgebühr zu entrichten. Damit gelten die Benennungsgebühren für **alle Vertragsstaaten des EPÜ**[1] als entrichtet (Art. 2 Nr. 3 GebO), soweit sie **in der internationalen Anmeldung** bestimmt sind[2].

☐ 10.2 Abweichend von der Erklärung in Nr. 10.1 ist derzeit beabsichtigt, **weniger als sieben** Benennungsgebühren für folgende **in der internationalen Anmeldung bestimmte Vertragsstaaten des EPÜ**[2] zu entrichten:

(1) ☐ _____
(2) ☐ _____
(3) ☐ _____

Soweit unter Nr. 10.2 Vertragsstaaten aufgeführt sind, wird beantragt, für die dort nicht aufgeführten Vertragsstaaten von der Zustellung einer Mitteilung nach Regel 108(3) EPÜ abzusehen.

☒ 10.3 Wird ein **automatischer Abbuchungsauftrag** erteilt (Feld 12), so wird das EPA beauftragt, bei Ablauf der Grundfrist nach Regel 107 (1)d) EPÜ den siebenfachen Betrag einer Benennungsgebühr abzubuchen. Ist eine Erklärung nach Nr. 10.2 abgegeben worden, so sollen die Benennungsgebühren nur für die dort angegebenen Vertragsstaaten abgebucht werden, sofern dem EPA nicht bis zum Ablauf der Grundfrist ein anderslautender Auftrag zugeht.

10. Designation fees

10.1 It is currently intended to pay **seven times** the amount of the designation fee. The designation fees for **all the EPC contracting states**[1] **designated in the international application**[2] are thereby deemed to have been paid (Art. 2 No. 3 RFees).

10.2 The declaration in No. 10.1 does not apply. Instead, it is currently intended to pay **fewer than seven** designation fees for the following **EPC contracting states**[2] **designated in the international application**:

(4) ☐ _____
(5) ☐ _____
(6) ☐ _____

If contracting states are indicated under No. 10.2, it is requested that no communication under Rule 108(3) EPC be issued for contracting states not thus indicated.

10.3 If an **automatic debit order** has been issued (Section 12), the EPO is authorised, on expiry of the basic period under Rule 107(1)(d) EPC, to debit seven times the amount of the designation fee. If states are indicated under No. 10.2, the EPO will debit designation fees only for those states, unless instructed otherwise before the basic period expires.

10. Taxes de désignation

10.1 Il est actuellement envisagé de payer un montant correspondant à **sept fois** la taxe de désignation. Les taxes de désignation sont ainsi réputées payées pour **tous les Etats contractants de la CBE**[1] **désignés dans la demande internationale**[2] (art. 2, point 3 du RRT).

10.2 Contrairement à ce qui est indiqué au n° 10.1, il est actuellement envisagé de payer **moins de sept** taxes de désignation pour les **Etats contractants de la CBE**[2] suivants **désignés dans la demande internationale** :

Si des Etats contractants sont mentionnés au n° 10.2, prière de ne pas procéder à la signification d'une notification prévue par la règle 108(3) CBE pour les Etats contractants n'y étant pas mentionnés.

10.3 Si un **ordre de prélèvement automatique** est donné (rubrique 12), il est demandé à l'OEB de prélever, à l'expiration du délai normal visé à la règle 107(1)d) CBE, un montant correspondant à sept fois la taxe de désignation. Si une déclaration a été faite au n° 10.2, les taxes de désignation ne sont à prélever que pour les Etats contractants qui y sont indiqués, sauf instruction contraire reçue par l'OEB avant l'expiration du délai normal.

1 Stand bei Drucklegung: 27 Vertragsstaaten, und zwar: / Status when this form was printed: 27 contracting states, namely / Situation à la date d'impression : 27 Etats contractants, à savoir : **AT** Österreich / Austria / Autriche, **BE** Belgien / Belgium / Belgique, **BG** Bulgarien / Bulgaria / Bulgarie, **CH / LI** Schweiz und Liechtenstein / Switzerland and Liechtenstein / Suisse et Liechtenstein, **CY** Zypern / Cyprus / Chypre, **CZ** Tschechische Republik / Czech Republic / République tchèque, **DE** Deutschland / Germany / Allemagne, **DK** Dänemark / Denmark / Danemark, **EE** Estland / Estonia / Estonie, **ES** Spanien / Spain / Espagne, **FI** Finnland / Finland / Finlande, **FR** Frankreich / France / France, **GB** Vereinigtes Königreich / United Kingdom / Royaume-Uni, **GR** Griechenland / Greece / Grèce, **HU** Ungarn / Hungary / Hongrie, **IE** Irland / Ireland / Irlande, **IT** Italien / Italy / Italie, **LU** Luxemburg / Luxembourg / Luxembourg, **MC** Monaco / Monaco / Monaco, **NL** Niederlande / Netherlands / Pays-Bas, **PT** Portugal / Portugal / Portugal, **RO** Rumänien / Romania / Roumanie, **SE** Schweden / Sweden / Suède, **SI** Slowenien / Slovenia / Slovénie, **SK** Slowakische Republik / Slovak Republic / République slovaque, **TR** Türkei / Turkey / Turquie

2 Für folgende Staaten nur möglich, falls in der internationalen Anmeldung am oder nach folgendem Tag bestimmt: Slowakische Republik, Bulgarien, Tschechische Republik und Estland: 1. Juli 2002, Slowenien: 1. Dezember 2002, Ungarn: 1. Januar 2003 und Rumänien: 1. März 2003. / For the following states this is possible only if they are designated in the international application on or after the stated date: Slovak Republic, Bulgaria, Czech Republic and Estonia: 1 July 2002, Slovenia: 1 December 2002, Hungary: 1 January 2003 and Romania: 1 March 2003. / En ce qui concerne les Etats suivants seulement si la désignation a été effectuée dans la demande internationale à la date suivante ou à une date ultérieure: République slovaque, Bulgarie, République tchèque et Estonie: 1er juillet 2002, Slovénie: 1er décembre 2002, Hongrie: 1er janvier 2003 et Roumanie: 1er mars 2003.

☒ **11. Erstreckung des europäischen Patents**
Bei Zahlung der Erstreckungsgebühr(en) gilt diese Anmeldung auch als wirksamer Erstreckungsantrag für die in der internationalen Anmeldung bestimmten »Erstreckungsstaaten«. Es ist beabsichtigt, diese Gebühr(en) für folgende Staaten zu entrichten:

11. Extension of the European patent
On payment of the extension fee(s) this application is also deemed to be a request for extension to all the "extension states" designated in the international application. It is intended to pay the fee(s) for the following states:

11. Extension des effets du brevet européen
La taxe (Les taxes) d'extension payée(s), la présente demande est également réputée être une demande d'extension à tous les «Etats autorisant l'extension» désignés dans la demande internationale. Il est envisagé de payer la taxe (les taxes) d'extension pour les Etats suivants:

☐	SI	Slowenien [1]	Slovenia [1]	Slovénie [1]
☐	LT	Litauen	Lithuania	Lituanie
☐	LV	Lettland	Latvia	Lettonie
☐	AL	Albanien	Albania	Albanie
☐	RO	Rumänien [1]	Romania [1]	Roumanie [1]
☐	MK	Ehemalige jugoslawische Republik Mazedonien	Former Yugoslav Republic of Macedonia	Ex-République yougoslave de Macédoine
☐	____	_____ [2]	_____ [2]	_____ [2]

1) Für Slowenien und Rumänien nur möglich, falls in der internationalen Anmeldung bis 30. November 2002 (Slowenien) oder bis 28. Februar 2003 (Rumänien) bestimmt. / For Slovenia and Romania this is possible only if they are designated in the international application up to 30 November 2002 (Slovenia) or 28 February 2003 (Romania). / En ce qui concerne la Slovénie et la Roumanie, seulement si la désignation a été effectuée dans la demande internationale jusqu'au 30 novembre 2002 (Slovénie) ou jusqu'au 28 février 2003 (Roumanie).

2) Platz für Staaten, mit denen »Erstreckungsabkommen« nach Drucklegung dieses Formblatts in Kraft treten und die in der internationalen Anmeldung bestimmt waren. / Space for States with which "extension agreements" enter into force after this form has been printed and which were designated in the international application. / Prévu pour des Etats à l'égard desquels des «accords d'extension» entreront en vigueur après l'impression du présent formulaire et qui ont été désignés dans la demande internationale.

☐ **12. Automatischer Abbuchungsauftrag** ***(Nur möglich für Inhaber von beim EPA geführten laufenden Konten)***

Das EPA wird beauftragt, nach Maßgabe der Vorschriften über das automatische Abbuchungsverfahren fällige Gebühren und Auslagen vom untenstehenden laufenden Konto abzubuchen. In Bezug auf die **Benennungsgebühren** wird auf Feld 10.3 verwiesen. Das EPA wird ferner beauftragt, die **Erstreckungsgebühren** für jeden in Feld 11 angekreuzten »Erstreckungsstaat« bei Ablauf der Grundfrist zu ihrer Zahlung abzubuchen, sofern ihm nicht bis dahin ein anderslautender Auftrag zugeht.

Nummer und Kontoinhaber

12. Automatic debit order ***(for EPO deposit account holders only)***

The EPO is hereby authorised, under the Arrangements for the automatic debiting procedure, to debit from the deposit account below any fees and costs falling due. For **designation fees**, see Section 10.3. The EPO is also authorised, on expiry of the basic period for paying the **extension fees**, to debit those fees for each of the "extension states" marked with a cross in Section 11, unless instructed otherwise before the said period expires.

Number and account holder

12. Ordre de prélèvement automatique ***(uniquement possible pour les titulaires de comptes courants ouverts auprès de l'OEB)***

Par la présente, il est demandé à l'OEB de prélever du compte courant ci-dessous les taxes et frais venant à échéance, conformément à la réglementation relative au prélèvement automatique. Pour les **taxes de désignation,** se reporter à la rubrique 10.3. Il est en outre demandé à l'OEB de prélever, à l'expiration du délai normal prévu pour leur paiement, les **taxes d'extension** pour chaque «Etat autorisant l'extension» coché à la rubrique 11, sauf instruction contraire reçue avant l'expiration de ce délai.

Numéro et titulaire du compte

☒ **13.** Eventuelle **Rückzahlungen** auf das beim EPA geführte laufende Konto

Nummer und Kontoinhaber

13. Any **reimbursement** to EPO deposit account

Number and account holder

2830.0297 - PIONEER

13. Remboursements éventuels à effectuer sur le compte courant ouvert auprès de l'OEB
Numéro et titulaire du compte

14. Unterschrift(en) des (der) Anmelder(s) oder Vertreters

Ort / Datum

Für Angestellte (Art. 133(3) EPÜ) mit allgemeiner Vollmacht:

Nr.

Name(n) des (der) Unterzeichneten bitte in Druckschrift wiederholen. Bei juristischen Personen bitte auch die Stellung des (der) Unterzeichneten innerhalb der Gesellschaft in Druckschrift angeben.

14. Signature(s) of applicant(s) or representative

BENTHAM, Andrew

Place / Date
London, GB, 4 November 2005
For employees (Art. 133(3) EPC) having a general authorisation:

No.

Please print name(s) under signature(s). In the case of legal persons, the position of the signatory within the company should also be printed.

14. Signature(s) du (des) demandeur(s) ou du mandataire

Lieu / Date

Pour les employés (art. 133(3) CBE) disposant d'un pouvoir général :

N°

Le ou les noms des signataires doivent être indiqués en caractères d'imprimerie. S'il s'agit d'une personne morale, la position occupée au sein de celle-ci par le ou les signataires doit également être indiquée en caractères d'imprimerie.

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EPO - Munich
85
07. Nov. 2005

The European Patent Office
Erhardtstrasse 27
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4 November 2005

Dear Sirs

European Patent Application No. 04751097.9
Regional Phase of International Application No. PCT/US04/13538
DOW AGROSCIENCES LLC *et al*
Our Ref: N.96314 AB/eod

I request entry of the above International Application into the European Regional Phase. I also request examination under Article 94 EPC. I enclose the following documents:

1. EPO Form 1200.

2. A debit order drawn on Pioneer Hi-Bred International, Inc.'s Deposit Account No. 2830.0297 for payment of the national fee, 7 designation fees (for Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Switzerland (and Liechtenstein), Germany, Denmark, Estonia, Spain, Finland, France, United Kingdom, Greece, Hungary, Ireland, Italy, Luxembourg, Monaco, Netherlands, Poland, Portugal, Romania, Slovak Republic, Slovenia, Sweden and Turkey), 31 excess claims fees, the examination fee and the third year renewal fee. The search fee is not being paid because the European Patent Office acted as the International Searching Authority. In the event of any deficiency or underpayment in the fees necessary for entry into the European Regional Phase, please deduct the necessary fees from the above-mentioned deposit account.

   As you are aware, European Patent Attorneys of J.A. Kemp & Co. are authorised to debit fees from Pioneer's deposit account on Pioneer's behalf.

3. A copy of new claims pages 45 to 49. These are to replace published pages 45 to 52.

4. A copy of each of old pages 49 to 52 showing the changes made to them.

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I am not filing Designation of Inventor forms because the Inventors were named in the International Application. If Designation of Inventor forms are required, please let me know. I request that EPO processing is carried out on the basis of the International Application as published except that pages 45 to 52 are replaced by pages 45 to 49 attached.

As the Examiner will appreciate, the amendments to the claims are simply editorial, and serve to bring the claim language used more into line with EPO practice.

Also, Inventor Tagliani is correctly included on the PCT coversheet but missing from the EPO's Register. Please correct this by adding her back.

Also, on the PCT coversheet, and hence also on the EPO's online Register, some of the state codes in the Inventors' addresses are incorrect:

- Inventor Cardineau's state is Arizona (AZ) (not Alabama (AL))
- Inventor Gupta's state is Indiana (IN) (not Idaho (ID))
- Inventor Hondred's state is Indiana (IN) (not Idaho (ID))
- Inventor Tagliani's state is Indiana (IN) (not Idaho (ID))

Please acknowledge receipt of this letter and the enclosures by stamping and returning the attached acknowledgement copy.

Yours faithfully

A. BENTHAM
AUTHORISED REPRESENTATIVE

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.
2. 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.
3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.
6. 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 or 27.
7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.
8. 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.
9. 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.

10. A plant comprising the DNA construct of claim 9.

11. A plant of claim 10, wherein said plant is a corn plant.

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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.

23. 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, or 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.

24. 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.

25. 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.

26. 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.

27. 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.

28. A descent plant of the insect resistant corn plant of claim 27 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.

29. Seed of a plant of claim 27 or 28.

30. A method of producing an insect resistant corn plant comprising breeding with a plant of claim 27 or 28, 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.

31. 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

35. The method of claim 34 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.

36. 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.

37. 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.

38. 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.

39. 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.

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

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

~~WHAT IS CLAIMED IS:~~ CLAIMS

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, or~~

~~5. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:27.~~

2. 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.

3. The kit of claim 2 further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.

4. The kit of claim 2 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.~~

5. The kit of claim 3, 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; or

~~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.

6. 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, or [PTO]

~~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, or

~~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.

8. 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.

9. 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.

10. A plant comprising the DNA construct of claim ~~20~~ 9.

11. A plant of claim ~~21~~ 10, wherein said plant is a corn plant.

12. 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.

13. The method of claim ~~23~~ 12, 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.

14. ~~25~~. The method of claim ~~24~~ 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15 ~~26~~. The method of claim ~~23~~ 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16 ~~27~~. The method of claim ~~25~~ 14, wherein said first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or

~~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~~ 15 the sequence of SEQ ID NO:3 and SEQ ID NO:5 respectively; or

~~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.

18 ~~31~~. The method of claim ~~27~~ 16, 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.~~

20 ~~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~~ or about 252 bp ~~using a TC1507 PCR identification protocol.~~

19 ~~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 , PTO (A) (B) (C);

(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 or 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 claim 36, 17.

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.

21

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.

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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.

23.

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.

24.

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.

25. 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.

26. 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.

27. 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.

28. A descent plant of the insect resistant corn plant of claim 27 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.

29. Seed of a plant of claim 27 or 28.

30. A method of producing an insect resistant corn plant comprising breeding with a plant of claim 27 or 28, 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.

31. 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or

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

34. 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.

35 60. The method of claim 34 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.

36 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.

37 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.

38 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.

39 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.

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

41
56. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

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EPO - Munich
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02. Dez. 2005

1 December 2005

Dear Sirs

European Patent Application No. 04751097.9
DOW AGROSCIENCES LLC *et al*
Our Ref: N.96314 AB/eod

Further to my letter of 4 November 2005 bringing this Application into the European Regional Phase, please will you make some further changes to the address details of the Inventors as follows.

- Inventor Barbour's street address is N.W. 97th Street rather than 118th Avenue.
- Inventor Hondred's state of residence is in fact Iowa (IA) rather than Indiana (IN) or Idaho (ID).

Please acknowledge receipt of this letter by stamping and returning the acknowledgement copy provided.

Yours faithfully



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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2405 PCT/US2004013538
Applicant/Proprietor	
Dow Agrosciences LLC, et al	

Communication pursuant to Rules 109 and 110 EPC

(1) Amendment of application documents, especially the claims (R. 109 EPC)

The above mentioned international (Euro-PCT) application has entered the European phase, or can do so, once the necessary conditions are fulfilled.

Under Articles 28, 41 PCT, Rules 52, 78 PCT and Rule 86(2) to (4) EPC, the applicant may amend the application documents after receiving the international search report.

Whether or not he has already done so, he now has a further opportunity to file amended claims or other application documents within a non-extendable time limit of one month after notification of the present communication (R. 109 EPC).

The claims applicable on expiry of the above time limit, i.e. those filed on entry into the European phase or in response to the present communication, will form the basis for the calculation of any claims fee to be paid (see page 2) and for any supplementary search to be carried out under Article 157(2) EPC (R. 109 EPC).

--2/

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(2) Claims fees under Rule 110 EPC

If the application documents on which the European grant procedure is to be based comprise more than ten claims, a claims fee shall be payable for the eleventh and each subsequent claim within the period provided for in Rule 107(1) EPC.

☑ Based on the application documents currently on file, all necessary claims fees have already been paid (or the documents do not comprise more than 10 claims).

☐ All necessary fees will be/have been debited automatically according to the automatic debit order.

☐ The claims fee due for the claims ..... to ..... were not paid within the above-mentioned period.

Any non-paid claims fee, either based on the current set of claims or on any amended claims to be filed pursuant to Rule 109 EPC (see page 1), may still be validly paid within a non-extendable period of grace of **one month** after notification of this communication.

If a payment is made for only some of the claims, it must be indicated for which claims it is intended. If a claims fee is not paid in due time, the claim concerned is deemed to be abandoned (R. 110(4) EPC).

If claims fees have already been paid, but on expiry of the above-mentioned time limit there is a new set of claims containing fewer fee-incurring claims than previously, the claims fees in excess of those due under Rule 110(2), 2nd sentence, EPC will be refunded (R. 110(3) EPC).

You are reminded that any supplementary search under Article 157(2) EPC will relate only to the last set of claims applicable on expiry of the above time limit AND will be confined to those fee-incurring claims for which fees have been paid in due time.

The fee for the eleventh and each subsequent claim is EUR 40,00.

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Reference	Application No./Patent No.
	04751097.9-2405-US2004013538
Applicant/Proprietor	
Dow Agrosciences LLC, et al	

Notification of the data mentioned in Article 128(5) EPC pursuant to Rule 17(3) EPC

In the above-identified patent application you are designated as inventor/co-inventor.
Pursuant to Rule 17(3) EPC the data as mentioned in Article 128(5) EPC are notified herewith:

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

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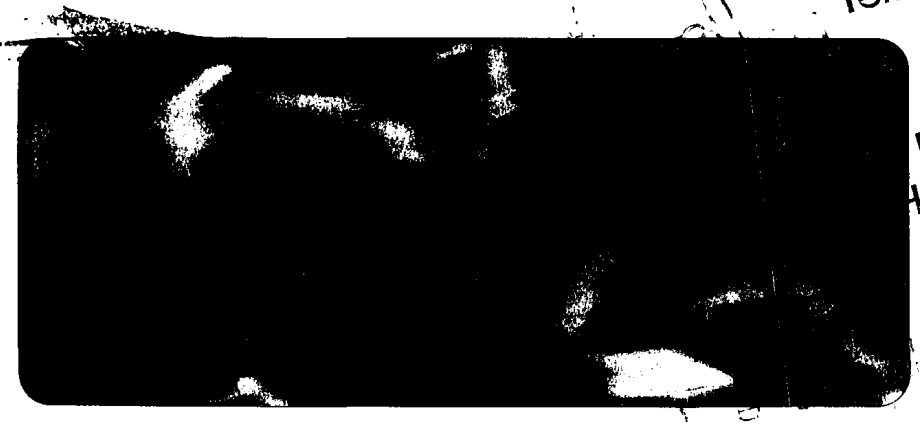
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Date
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Reference	Application No./Patent No.
	04751097.9-2405-US2004013538
Applicant/Proprietor	
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Date
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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2405 PCT/US2004013538

Applicant/Proprietor
Dow Agrosciences LLC, et al

Notification of European publication number and information on the application of Article 67(3) EPC

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 details, see information brochure of the European Patent Office "National Law relating to the EPC" and additional information in the Official Journal of the European Patent Office).

Pursuant to Article 158(1) EPC the publication under Article 21 PCT of an international application for which the European Patent Office is a designated Office takes the place of the publication of a European patent application.

The bibliographic data of the above-mentioned Euro-PCT application will be published on 01.02.06 in Section I.1 of the European Patent Bulletin. The European publication number is 1620571.

In all future communications to the European Patent Office, please quote the application number plus Directorate number.

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Reference	Application No./Patent No.
	04751097.9-2405-US2004013538
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Notification of the data mentioned in Article 128(5) EPC pursuant to Rule 17(3) EPC

In the above-identified patent application you are designated as inventor/co-inventor.
Pursuant to Rule 17(3) EPC the data as mentioned in Article 128(5) EPC are notified herewith:

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PRIORITY	: US/02.05.03/ USP 467772
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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

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Reference	Application No./Patent No.
	04751097.9-2405-US2004013538
Applicant/Proprietor	
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Notification of the data mentioned in Article 128(5) EPC pursuant to Rule 17(3) EPC

In the above-identified patent application you are designated as inventor/co-inventor.
Pursuant to Rule 17(3) EPC the data as mentioned in Article 128(5) EPC are notified herewith:

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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402
Applicant/Proprietor: Dow Agrosciences LLC, et al	

Notification pursuant to part A-III, 5.3 of the Guidelines for Examination in the EPO

The communication issued pursuant to Rule 17(3) EPC, sent to the inventor designated below, has been returned by the postal services.
You are requested to indicate the correct address of the inventor (R. 17(1) EPC).

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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402
Applicant/Proprietor: Dow Agrosciences LLC, et al	

Notification pursuant to part A-III, 5.3 of the Guidelines for Examination in the EPO

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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402
Applicant/Proprietor: Dow Agrosciences LLC, et al	

Notification pursuant to part A-III, 5.3 of the Guidelines for Examination in the EPO

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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402
Applicant/Proprietor: Dow Agrosciences LLC, et al	

Notification pursuant to part A-III, 5.3 of the Guidelines for Examination in the EPO

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06. März 2006

2 March 2006

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC *et al.*
Our Ref: N.96314 AB/MJG/nw

I refer to the four Invitations dated 24 and 30 January 2006. Inventor VAN ZANTE's current address is the same as that listed on the Invitation. The correct addresses for the three remaining Inventors are listed below:

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Yours faithfully

A BENTHAM

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Application No.	Ref.	Date
04 751 097.9 - 2402	N.96314 AB	09.03.2006
Applicant: Dow Agrosciences LLC		

Communication pursuant to Article 96(2) 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(1) 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 78(2) and 83(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 (Rule 36(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Article 96(3) EPC).

Helliot, B
Primary Examiner
for the Examining Division

Enclosure(s): 3 page/s reasons (Form 2906)

Registered Letter
EPO Form 2001 07.02CSX

The examination is being carried out on the following application documents:

Description, Pages

1-44 as published

Sequence listings part of the description, Pages

1-21 as published

Claims, Numbers

1-41 received on 07.11.2005 with letter of 04.11.2005

Drawings, Sheets

1/1 as published

1. INTRODUCTION

The following documents (D1-D4) 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, [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].

D2: 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].

D2': 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.

D3: US 2002/162136 A1 (HOHN THOMAS M ET AL) 31 October 2002 (2002-10-31).

D4: 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 present application relates to the insect resistant corn (Zea mays) plant TC1507, to the DNA plant expression construct of corn plant TC1507 and to the detection of the transgene/flanking insertion region in corn plant TC1507 and progeny thereof.

2. AMENDMENTS (Art. 123(2) EPC)

The amendments filed with the letter dated November, 7th 2005, do not introduce subject-matter which extends beyond the content of the application as filed, and therefore meet the requirement of Article 123(2) EPC.

3. REMARK

An international preliminary examination report has already been drawn up for the present application in accordance with the PCT. The deficiencies mentioned in that report give rise to objections under the corresponding provisions of the EPC.

4. CONCLUDING REMARKS

a. 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 29(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.

b. 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).

c. To meet the requirements of Rule 27(1)(b) EPC, the documents D1, D2 and D3, would have to be identified in the description and the relevant background art disclosed therein would have to be briefly discussed.

d. **The amendments would have to be filed taking into account the requirements of R. 36(1) 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.**

e. If amended claims are submitted, attention should be taken to the requirement of R.29(2) EPC with respect to the number of independent claims.

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Date
05.07.06

Reference	Application No./Patent No.
N.96314 AB	04751097.9-2402

Applicant/Proprietor
Dow AgroSciences LLC, et al

Refund of fees

The following fee was paid in respect of the application 04751097.9:

Fee	Code	Voucher No	Date	Currency	Amount
	093	00394255	30.05.06	EUR	40,00

According to the present state of the file the refund will be made by:

CREDITING YOUR DEPOSIT ACCOUNT 28300297.

Amount refundable:	Code	Currency	Amount	Voucher No
	093	EUR	40,00	00434776

Reason for refund: Undue payment.

The Authorising Officer
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Reference	Application No./Patent No.
N.96314 AB	04751097.9-2402

Applicant/Proprietor
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Refund of fees

The following fee was paid in respect of the application 04751097.9:

Fee	Code	Voucher No	Date	Currency	Amount
Renewal fee	033	00648730	07.11.05	EUR	380,00
Renewal fee	033	00394255	30.05.06	EUR	400,00

According to the present state of the file the refund will be made by:

CREDITING YOUR DEPOSIT ACCOUNT 28300297.

Amount refundable:	Code	Currency	Amount	Voucher No
	033	EUR	400,00	00434776

Reason for refund: Fee paid twice.

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BY DHL COURIER

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59
06. Juli 2006

5 July 2006

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC
Our Ref: N.96314 AB/eod

I refer to your Communication of 9 March 2006 and request a two month extension to the period for response, taking the total response period to six months. Please confirm in due course that this extension has been granted.

In the meantime, please acknowledge receipt of this letter by stamping and returning the attached acknowledgment copy.

Yours faithfully

A. BENTHAM
AUTHORISED REPRESENTATIVE

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Date 13.07.06

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402

Applicant/Proprietor
Dow AgroSciences LLC, et al

Extension of time limit pursuant to Rule 84 EPC

Examination procedure

With reference to your request, the time limit for replying to the communication dated 09.03.06 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, part E-VIII, 1.6.

If no reply to the communication is received in due time, the European patent application will be deemed to be withdrawn (Article 96(3) EPC).

Examining Division

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OUR REF.: N.96314 AB/eod

DATE: 19 September 2006

YOUR REF.: 04751097.9-2402

NO. OF PAGES: 15
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Please would you acknowledge safe and complete receipt of this transmission in legible form by signing and returning this sheet to us by fax

SIGNED: _____ DATE: _____

Received at the EPO on Sep 19, 2006 18:32:44. Page 1 of 15

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19 September 2006

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC
Our Ref: N.96314 AB/eod

This is in response to the Examination Report dated 9 March 2006. Please find attached a new set of claims, in hand-amended and retyped formats, to replace the current claims.

The points in the Examination Report itself call for no particular comment, but the Applicant's submissions on the issues raised by the EPO as ISA in item V.2 of its report are set out below.

AMENDMENTS

Claims 9, 10 and 11 have been cancelled. To avoid complex renumbering, claims 37, 39 and 40 have been renumbered as new claims 9, 10 and 11. Claim 27 has been cancelled, and the relevant language from it moved to claim 30 (new claim 27). New claims 28 to 30 have been converted to method format and rendered dependent on new claim 27 (previous claim 30). Claim 31 has also been amended. The dependency of claim 39 (new claim 37) has also been corrected.

It is proposed to hold in abeyance any amendments to the description until agreement is reached on a set of claims.

Items (c), (n) and (j) have been addressed by amendment, i.e. by cancellation of claims 9 to 11 (claims 20-22 as published), by the changes to claims 27-30 (claims 31 to 54 as published), and by removal of reference to SEQ ID NO: 1 from claim 31.

NOVELTY

Items (i), (j) and (l) all concern novelty, over D1, D3 and D4 respectively.

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D1 does not take away the novelty of claim 53 as published (present claim 21). To take away novelty, a citation must disclose, clearly and unambiguously, all the features of the claim. In as much as it refers to Southern blotting (see D1, page 2, first paragraph below "Characteristics of the modification: the introduced DNA"), D1 does not disclose the features of current claim 21. There is no clear and ambiguous disclosure of the use of a probe that hybridises under stringent hybridisation conditions with DNA from maize event TC1507 and not with DNA from a non-TC1507 maize plant. In fact, there is no disclosure of hybridisation conditions at all.

Moreover, so far as Southern blotting is concerned, D1 provides no enabling disclosure. Based on D1, the skilled person does not know what probe to use. As will be discussed in more detail below, D1 provides no information as to the sequence of the crucial junction regions involved in the TC1507 event. Therefore, the skilled person seeking to practice the purported disclosure regarding Southern blotting does not have the tools with which to work. By contrast, the junction sequences are provided in the present Application so that the skilled person seeking to practice the method of claim 21 does have the necessary tools.

Related to this, Southern blotting is a fairly crude technique, and it is not clear from D1 whether the detection method of D1 was specific to the TC1507 event, as required by the claims, or generic to any transgenic insertion of the relevant DNA. Therefore, there is no clear and unambiguous disclosure of an event-specific method as required herein (see below for further discussion of the meaning of "event" and event-specificity).

Regarding item (j) and D3, claim 31 has been amended to expedite examination. However, regarding claim 22, bear in mind that SEQ ID NO: 1 in D3 is 1,403 nucleotides in length. It may be the case that the 30-nucleotide sequence of present SEQ ID NO: 1 can be found somewhere within SEQ ID NO: 1 of D3 but this does not take away novelty of present claim 22 (claims 44/45 and 55 as filed). Claim 22 requires "an isolated DNA primer sequence". The long sequence of SEQ ID NO: 1 of D3 is surely not suitable as a primer.

Regarding item (l) and claim 24 (claim 48 as filed), attention is drawn to the term "isolated" in claim 24. With respect, it is artificial and unrealistic to suggest that the sequence of an entire mouse chromosome of 194,175 nucleotides can take away the novelty of a short primer of 20 nucleotides in length. It is true that claim 24 uses the generally "open" term "comprising", but this has to be read sensibly, as the skilled person would understand it, and in conjunction with "isolated". An enormous sequence such as that of a chromosome is by no means "isolated" in the sense of the claim.

INVENTIVE STEP

The remaining issues all relate to inventive step and can be dealt with together.

D1 and D2' do nothing to take away inventive step of the present claims. Fundamentally, the Examining Division's point was that TC1507 and the PHI8999A transgenic casette were known from D1, such that the present subject matter was said to be obvious over D1/D2. In fact, when the invention is fully understood, it is clear that there is no lack of inventive step.

The invention pertains to the detection of the TC1507 "event" in maize. The term "event" refers to a specific integration of transgene into a plant at a specific, initially unknown, genetic locus. The same plant could be transformed many times with the same transgene and this would represent many different events because the transgene would incorporate in different places and orientations, and also because more or less flanking sequence from the transformation construct might be lost, duplicated, rearranged etc during each transformation.

Any given event can be transmitted by breeding from the original transformant plant, thereby to create descendent plants with the same event. The present invention is about detecting one particular event, known as TC1507, in maize. Having understood what an event is, it can be seen that part of what distinguishes one event from another is the flanking sequence at either end of the insert. This is distinctive because it contains partly inserted construct sequence and partly original plant sequence. Therefore, if one imagines two different events with the same transgene, the "junction" sequences between the inserted DNA and the native genomic DNA will be different because (a) the transgene is in two different genomic locations, and possibly in different orientations, and (b) different losses, duplications, rearrangements etc of nucleotides will have occurred.

To detect the TC1507 in maize, the present invention relies on probe/primer sequences designed to hybridise with and/or amplify the unique junction sequences of TC1507. These are the sequences given in the claims (any claims not relating to junction-specific probe/primer/hybridisation/ amplification having been cancelled). To design these primers, it was necessary for the Inventors to locate and identify the transgenic insert in TC1507 maize.

In item (b) of its comments, the ISA stated that "sequences methods of flanking regions are routinely used by the skilled person to confirm and identify the incorporation site of a transgenic set". However, no documentary support was provided for that proposition and, in fact, I understand from the Applicant that identifying transgenic events in maize is not a matter of routine. Furthermore, the identification of TC1507 in particular, leading to the design of the junction sequence, event-specific primers of the invention, was particularly challenging.

TC1507 was a biolistic event, in other words the PHI8999 construct was shot into the tissue at high velocity by microprojectile bombardment. The sequence of the introduced plasmid was known, but nothing was known about, for example, the orientation of the resultant insert, whether or not and if so to what extent, it had been fragmented and/or truncated, or its location in the plant genome. Broadly, two steps were thus required. First, the insert had to be located, and this required an entire gene discovery project in its own right. Second, to design primers that were event-specific, not just construct-specific or gene-specific, the event had to be defined in terms of the sequences at the junctions between the inserted DNA and the native genomic DNA. In general, such exercises are more difficult where there are repeated, duplicated sequences, because this introduces difficulties with secondary structures.

In this particular case, multiple genome walking, genomic library, hybridization and data analysis methods were used to isolate, sequence and assemble the unknown regions. The process was not trivial and numerous techniques were necessary due to the unique structure (truncated, inverted, repeated and rearranged fragments) and repetitive nature of areas of the insert and surrounding

genomic DNA. Final confirmation of the discovered sequence required intensive direct PCR cloning and sequence of regions that were highly recalcitrant to replication in *E. coli* that is commonly used for gene cloning. Standard sequence assembly programs were not capable of accurately analyzing and assembling the repeated sequences and intensive manual curation was necessary for final assembly. Identification and final assembly of the region was as challenging as traditional cloning of unique previously unknown and uncharacterized genome regions with the additional complexity of fully sequencing repetitive regions that are generally not completely characterized in genome sequencing projects due to the difficulty involved.

The exact sequence of the insertion region and surrounding maize genomic DNA was a prerequisite to developing an event specific PCR detection method. Initial known sequence of the PHP8999A fragment would not provide sufficient information for a PCR detection method to uniquely identify the insertion in 1507 maize. Event specific detection methods require the design of primers spanning unique junctions formed upon the insertion of the DNA into the maize genomic DNA forming novel junctions with the genomic DNA or other novel junctions due to the fragmentation and rearrangement of the original DNA. These unique junctions were only identified and made available following the discovery process described above.

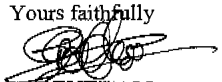
In order to develop the event specific detection method for 1507 maize that met stringent validation requirements, it was necessary to test primers across more than one unique junction region. As with the cloning process, certain regions of the DNA sequence formed due to the insertion or endogenous to the maize genomic sequence in the insert region were prohibitive to successful PCR method validation. Both the identification and characterization of the insertion region and multiple unique junctions and proper design and validation of appropriate PCR methods were required for development of an event specific detection method and no part of this process was ordinary, obvious, or routine. It required not only a great deal of hard work, but also creative input to surmount the various difficulties outlined above. Only in this way was it possible to derive the probe/primer sequences of the invention and render it possible to detect the TC1507 event in maize.

For these reasons, it is submitted that an inventive step is present and Article 56 EPC is complied with.

I believe that the issues raised so far have now been addressed and I look forward to hearing from the Examining Division again in due course. If there are any minor points outstanding that the Examiner feels could usefully be discussed by telephone, he/she is welcome to call me to discuss those. If further substantive points remain, then I request that another Examination Report be issued. As a precaution, in the event of that the Examining Division contemplates refusing the Application without issuing another written Communication, Oral Proceedings are requested.

Please also acknowledge receipt of this letter by stamping and returning the acknowledgement copy provided.

Yours faithfully



A. BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.
2. 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.
3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.
6. 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 or 27.
7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.
8. 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.
9. 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.
10. 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.

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11. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.
12. 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.
13. The method of claim 12, 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.
14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.
15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.
16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.
17. The method of claim 15, wherein said first and second primers comprise: 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.
18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.
19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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

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(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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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.

23. 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, or 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.

24. 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.

25. 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.

26. 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.

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27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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,

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.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, 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 descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, 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 seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, 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 descent plant's genome.

31. 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: 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

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

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(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.

35. The method of claim 34 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.

36. 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.

37. The DNA detection kit of claim 36 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.

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

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CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.
2. 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.
3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.
6. 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 or 27.
7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.
8. 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.
9. ~~A DNA construct comprising: a first and second expression cassette, wherein said first expression cassette in operable linkage comprises~~ (SEE PAGE 49 FOR NEW CLAIMS 9-11)
   - ~~(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~~

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~~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.~~

~~10. A plant comprising the DNA construct of claim 9.~~

~~11. A plant of claim 10, wherein said plant is a corn plant.~~

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or SEQ ID NO:4 and SEQ ID NO:5;

(c) providing DNA amplification reaction conditions;

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(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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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.

23. 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, or 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.

24. 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.

25. 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.

26. 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

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recognizes a sequence within SEQ ID NO:21 or SEQ ID NO:22 in a sample of a seed lot.

27. ~~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,

28. A descent plant of ~~the~~ said insect resistant corn plant of claim 27, 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.

29. ~~Seed of a plant of claim 27 or 28.~~

30. ~~A~~ A method of producing an insect resistant corn plant comprising breeding with a plant, ~~of claim 27 or 28,~~ 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.

31. 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: 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

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35. The method of claim 34 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.

36. 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.

9 ~~37~~. 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.

10 ~~38~~. 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.

37 ~~39~~. The DNA detection kit of claim 36 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.

11 ~~40~~. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

38 ~~41~~. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

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19 September 2006

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC
Our Ref: N.96314 AB/eod

This is in response to the Examination Report dated 9 March 2006. Please find attached a new set of claims, in hand-amended and retyped formats, to replace the current claims.

The points in the Examination Report itself call for no particular comment, but the Applicant's submissions on the issues raised by the EPO as ISA in item V.2 of its report are set out below.

AMENDMENTS

Claims 9, 10 and 11 have been cancelled. To avoid complex renumbering, claims 37, 39 and 40 have been renumbered as new claims 9, 10 and 11. Claim 27 has been cancelled, and the relevant language from it moved to claim 30 (new claim 27). New claims 28 to 30 have been converted to method format and rendered dependent on new claim 27 (previous claim 30). Claim 31 has also been amended. The dependency of claim 39 (new claim 37) has also been corrected.

It is proposed to hold in abeyance any amendments to the description until agreement is reached on a set of claims.

Items (c), (n) and (j) have been addressed by amendment, i.e. by cancellation of claims 9 to 11 (claims 20-22 as published), by the changes to claims 27-30 (claims 31 to 54 as published), and by removal of reference to SEQ ID NO: 1 from claim 31.

NOVELTY

Items (i), (j) and (l) all concern novelty, over D1, D3 and D4 respectively.

D1 does not take away the novelty of claim 53 as published (present claim 21). To take away novelty, a citation must disclose, clearly and unambiguously, all the features of the claim. In as much as it refers to Southern blotting (see D1, page 2, first paragraph below "Characteristics of the modification: the introduced DNA"), D1 does not disclose the features of current claim 21. There is no clear and ambiguous disclosure of the use of a probe that hybridises under stringent hybridisation conditions with DNA from maize event TC1507 and not with DNA from a non-TC1507 maize plant. In fact, there is no disclosure of hybridisation conditions at all.

Moreover, so far as Southern blotting is concerned, D1 provides no enabling disclosure. Based on D1, the skilled person does not know what probe to use. As will be discussed in more detail below, D1 provides no information as to the sequence of the crucial junction regions involved in the TC1507 event. Therefore, the skilled person seeking to practice the purported disclosure regarding Southern blotting does not have the tools with which to work. By contrast, the junction sequences are provided in the present Application so that the skilled person seeking to practice the method of claim 21 does have the necessary tools.

Related to this, Southern blotting is a fairly crude technique, and it is not clear from D1 whether the detection method of D1 was specific to the TC1507 event, as required by the claims, or generic to any transgenic insertion of the relevant DNA. Therefore, there is no clear and unambiguous disclosure of an event-specific method as required herein (see below for further discussion of the meaning of "event" and event-specificity).

Regarding item (j) and D3, claim 31 has been amended to expedite examination. However, regarding claim 22, bear in mind that SEQ ID NO: 1 in D3 is 1,403 nucleotides in length. It may be the case that the 30-nucleotide sequence of present SEQ ID NO: 1 can be found somewhere within SEQ ID NO: 1 of D3 but this does not take away novelty of present claim 22 (claims 44/45 and 55 as filed). Claim 22 requires "an isolated DNA primer sequence". The long sequence of SEQ ID NO: 1 of D3 is surely not suitable as a primer.

Regarding item (l) and claim 24 (claim 48 as filed), attention is drawn to the term "isolated" in claim 24. With respect, it is artificial and unrealistic to suggest that the sequence of an entire mouse chromosome of 194,175 nucleotides can take away the novelty of a short primer of 20 nucleotides in length. It is true that claim 24 uses the generally "open" term "comprising", but this has to be read sensibly, as the skilled person would understand it, and in conjunction with "isolated". An enormous sequence such as that of a chromosome is by no means "isolated" in the sense of the claim.

INVENTIVE STEP

The remaining issues all relate to inventive step and can be dealt with together.

D1 and D2' do nothing to take away inventive step of the present claims. Fundamentally, the Examining Division's point was that TC1507 and the PHI8999A transgenic casette were known from D1, such that the present subject matter was said to be obvious over D1/D2. In fact, when the invention is fully understood, it is clear that there is no lack of inventive step.

The invention pertains to the detection of the TC1507 "event" in maize. The term "event" refers to a specific integration of transgene into a plant at a specific, initially unknown, genetic locus. The same plant could be transformed many times with the same transgene and this would represent many different events because the transgene would incorporate in different places and orientations, and also because more or less flanking sequence from the transformation construct might be lost, duplicated, rearranged etc during each transformation.

Any given event can be transmitted by breeding from the original transformant plant, thereby to create descendent plants with the same event. The present invention is about detecting one particular event, known as TC1507, in maize. Having understood what an event is, it can be seen that part of what distinguishes one event from another is the flanking sequence at either end of the insert. This is distinctive because it contains partly inserted construct sequence and partly original plant sequence. Therefore, if one imagines two different events with the same transgene, the "junction" sequences between the inserted DNA and the native genomic DNA will be different because (a) the transgene is in two different genomic locations, and possibly in different orientations, and (b) different losses, duplications, rearrangements etc of nucleotides will have occurred.

To detect the TC1507 in maize, the present invention relies on probe/primer sequences designed to hybridise with and/or amplify the unique junction sequences of TC1507. These are the sequences given in the claims (any claims not relating to junction-specific probe/primer/hybridisation/ amplification having been cancelled). To design these primers, it was necessary for the Inventors to locate and identify the transgenic insert in TC1507 maize.

In item (b) of its comments, the ISA stated that "sequences methods of flanking regions are routinely used by the skilled person to confirm and identify the incorporation site of a transgenic set". However, no documentary support was provided for that proposition and, in fact, I understand from the Applicant that identifying transgenic events in maize is not a matter of routine. Furthermore, the identification of TC1507 in particular, leading to the design of the junction sequence, event-specific primers of the invention, was particularly challenging.

TC1507 was a biolistic event, in other words the PHI8999 construct was shot into the tissue at high velocity by microprojectile bombardment. The sequence of the introduced plasmid was known, but nothing was known about, for example, the orientation of the resultant insert, whether or not and if so to what extent, it had been fragmented and/or truncated, or its location in the plant genome. Broadly, two steps were thus required. First, the insert had to be located, and this required an entire gene discovery project in its own right. Second, to design primers that were event-specific, not just construct-specific or gene-specific, the event had to be defined in terms of the sequences at the junctions between the inserted DNA and the native genomic DNA. In general, such exercises are more difficult where there are repeated, duplicated sequences, because this introduces difficulties with secondary structures.

In this particular case, multiple genome walking, genomic library, hybridization and data analysis methods were used to isolate, sequence and assemble the unknown regions. The process was not trivial and numerous techniques were necessary due to the unique structure (truncated, inverted, repeated and rearranged fragments) and repetitive nature of areas of the insert and surrounding

genomic DNA. Final confirmation of the discovered sequence required intensive direct PCR cloning and sequence of regions that were highly recalcitrant to replication in *E. coli* that is commonly used for gene cloning. Standard sequence assembly programs were not capable of accurately analyzing and assembling the repeated sequences and intensive manual curation was necessary for final assembly. Identification and final assembly of the region was as challenging as traditional cloning of unique previously unknown and uncharacterized genome regions with the additional complexity of fully sequencing repetitive regions that are generally not completely characterized in genome sequencing projects due to the difficulty involved.

The exact sequence of the insertion region and surrounding maize genomic DNA was a prerequisite to developing an event specific PCR detection method. Initial known sequence of the PHP8999A fragment would not provide sufficient information for a PCR detection method to uniquely identify the insertion in 1507 maize. Event specific detection methods require the design of primers spanning unique junctions formed upon the insertion of the DNA into the maize genomic DNA forming novel junctions with the genomic DNA or other novel junctions due to the fragmentation and rearrangement of the original DNA. These unique junctions were only identified and made available following the discovery process described above.

In order to develop the event specific detection method for 1507 maize that met stringent validation requirements, it was necessary to test primers across more than one unique junction region. As with the cloning process, certain regions of the DNA sequence formed due to the insertion or endogenous to the maize genomic sequence in the insert region were prohibitive to successful PCR method validation. Both the identification and characterization of the insertion region and multiple unique junctions and proper design and validation of appropriate PCR methods were required for development of an event specific detection method and no part of this process was ordinary, obvious, or routine. It required not only a great deal of hard work, but also creative input to surmount the various difficulties outlined above. Only in this way was it possible to derive the probe/primer sequences of the invention and render it possible to detect the TC1507 event in maize.

For these reasons, it is submitted that an inventive step is present and Article 56 EPC is complied with.

I believe that the issues raised so far have now been addressed and I look forward to hearing from the Examining Division again in due course. If there are any minor points outstanding that the Examiner feels could usefully be discussed by telephone, he/she is welcome to call me to discuss those. If further substantive points remain, then I request that another Examination Report be issued. As a precaution, in the event of that the Examining Division contemplates refusing the Application without issuing another written Communication, Oral Proceedings are requested.

Please also acknowledge receipt of this letter by stamping and returning the acknowledgement copy provided.

Yours faithfully

A. BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.
2. 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.
3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.
6. 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 or 27.
7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.
8. 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.
9. 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.
10. 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.

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

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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.

23. 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, or 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.

24. 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.

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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,

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.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, 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 descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, 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 seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, 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 descent plant's genome.

31. 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: 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

35. The method of claim 34 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.

36. 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.

37. The DNA detection kit of claim 36 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.

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

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.
2. 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.
3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.
6. 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 or 27.
7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.
8. 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.
9. ~~A DNA construct comprising: a first and second expression cassette, wherein said first expression cassette in operable linkage comprises~~ (SEE PAGE 49 FOR NEW CLAIMS 9-11)
   ~~(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.~~

~~10. A plant comprising the DNA construct of claim 9.~~

~~11. A plant of claim 10, wherein said plant is a corn plant.~~

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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.

23. 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, or 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.

24. 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.

25. 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.

26. 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.

~~27. 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,

28. A descent plant of ~~the~~ said insect resistant corn plant of claim 27, 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 descent plant's genome.

~~29. Seed of a plant of claim 27 or 28.~~

27 ~~30~~ A method of producing an insect resistant corn plant comprising breeding with a plant, ~~of claim 27 or 28,~~ 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.

31. 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

35. The method of claim 34 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.

36. 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.

9 ~~37~~. 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.

10 ~~38~~. 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.

37 ~~39~~. The DNA detection kit of claim 36 ~~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.

11 ~~40~~. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.

38 ~~41~~. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

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Application No.	Ref.	Date
04 751 097.9 - 2402	N.96314 AB	09.01.2007
Applicant: Dow AgroSciences LLC		

Communication pursuant to Article 96(2) 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(1) 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 78(2) and 83(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 (Rule 36(1) EPC).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Article 96(3) EPC).

Helliot, Bertrand
Primary Examiner
for the Examining Division

Enclosure(s): 5 page/s reasons (Form 2906)

Registered Letter
EPO Form 2001 07.02CSX

The examination is being carried out on the following application documents:

Description, Pages

1-44 as published

Sequence listings part of the description, Pages

1-21 as published

Claims, Numbers

1-38 received on 21.09.2006 with letter of 19.09.2006

Drawings, Sheets

1/1 as published

1. INTRODUCTION

When drafting this communication, the Examining Division has taken the arguments provided by the Applicant in his reply of September, 19th 2006, into consideration.

2. AMENDMENTS (Art. 123(2) EPC)

The amendments filed with the letter dated September, 19th 2006 do not introduce subject-matter which extends beyond the content of the application as filed, and therefore meet the requirement of Article 123(2) EPC.

3. CLARITY (Art. 84 EPC)

a. Present **claim 9** relates to a DNA kit comprising a polynucleotide probe that hybridizes under stringent conditions with one or more DNA sequences selected from the group consisting of SEQ ID N° 45-57 and complements thereof.
However, the alignment analysis performed between the said molecules and the molecule listed as SEQ ID N° 24 shows that the molecules listed as SEQ ID N° 48 and 53 do not have 100% identity with the molecule listed as SEQ ID N° 24. In view of that and since the said molecules listed as SEQ ID N° 48 and 53 would not hybridize under the claimed stringent hybridization conditions, present **claim 9** lacks therefore, clarity in the sense of Art. 84 EPC.

b. Present **claim 24** relates to an isolated DNA molecule comprising a junction sequence comprising a sequence selected from the group consisting of SEQ ID N° 45-57 and complements thereof.
However, the sequence alignment analysis performed between the nucleic acid molecule SEQ ID N° 24 and the claimed sequence shows that only the molecules listed as SEQ ID N° 52 and 53 hybridize to a junction sequence. Since the molecules listed as SEQ ID N° 45-51 and 54-57 do not hybridize to the junction sequences, present **claim 24** lacks, therefore, clarity in the sense of Art. 84 EPC.

c. **Claim 37** relates to the DNA detection kit of claim 36. However, the said **claim 37** cannot be dependent of claim 36 since the latter relates to a method for detecting the presence of the TC1507 event.

4. NOVELTY and INVENTIVE STEP (Art. 52(1), 54(1)(2) and 56 EPC)

a. After reconsideration, the Examining Division is of the opinion that the present application does not meet the requirements of Art. 52(1) EPC, because the subject-matter of **claim 1** is not new in the sense of Art. 54(1) and (2) EPC.

Present **claim 1** relates to an isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID N° 21, 22, 24, 26 or 27.
According to the Guidelines (see G.L., C-III, 4.13), the term "comprising" has to

be interpreted by the broad meaning "including", "containing" or comprehending". In view of that, and since the molecules listed as SEQ ID N° 21 and 22 do not hybridize to the junction sequences, *per se*, but to sequences of maize (see Tab. 2 and 7), the isolated DNA molecules comprising the SEQ ID N° 21 or 22 cannot be seen as novel since as formulated, the said claim encompasses the whole genome of maize.

In the case wherein the reference to the sequence SEQ ID N° 21 and 22 would be deleted from the present **claim 1**, the Applicant is informed that a such amended claim would be formally considered as novel in the sense of Art. 54(1) and (2) EPC.
Thus, even if D1 relates to the same transgenic maize line TC1507, the said document is silent as to any nucleic acid sequence of the transgenic event. Therefore, an isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID N° 24, 26 or 27 would be seen as novel (Art. 54(1)(2) EPC).

However, the Examining Division is of the opinion that the subject-matter of **claim 1** would not involve an inventive step over the disclosure of D1 (Art. 52(1) and 56 EPC).
The subject-matter of **claim 1** differs from that of D1 in that isolated DNA molecules are claimed.
However, the Applicant defines the term "event" as "*a specific integration of transgene into a plant at a specific, initially unknown, genetic locus*" (see letter p. 3).
Since D1 relates to the same transgenic maize line TC1507 as that one disclosed in the present application, the said maize line TC1507 of D1 results from the same TC1507 event to that one described in the present application. The nucleic acid sequences corresponding to the PH18999 construct as well as that one of the flanking sequences are considered to be the same by the Examining Division.
Moreover, in the absence of any additional technical effect linked to the particular claimed DNA molecules, the event specified in claim 1 is merely a non inventive alternative to the one disclosed in D1 that can be routinely generated by the skilled person.
In view of that and in the case wherein the reference to the sequence SEQ ID N° 21 and 22 would be deleted from the present **claim 1**, the Applicant is

informed that a such amended claim could not be considered as involving an inventive step in the sense of Art. 56 EPC.

b. No cited prior-art document discloses kits suitable for identifying the event TC1507 in a biological sample as that one disclosed in **claims 2-11, 37**. Therefore, the subject-matter of **claims 2-11, 37** is considered novel in the sense of Art. 54(1) and (2) EPC.

However, in the light of the objections put forward under item 4-a herein above, and since the present description is silent as to any additional technical effect linked to the said kits, **claims 2-11, 37** do not meet the requirements of Article 56 EPC because, for the person skilled in the art, grouping the materials used for a series of known or obvious experiments in the form of a kit is not inventive.

c. No cited prior-art document discloses methods for identifying/detecting the event TC1507 in a biological sample as that ones disclosed in **claims 12-19, 21, 25-36 and 38**.
Therefore, the subject-matter of **claims 12-19, 21, 25-36 and 38** is considered novel in the sense of Art. 54(1) and (2) EPC.

However, for the same reasons as set out under item 4-a herein above and since the present description is silent as to any additional technical effect linked to the said methods, **claims 12-19, 21, 25-36 and 38** do not appear inventive because detection methods based on nucleic acids relate to routine techniques at the date of the claimed priority (Article 56 EPC).

d. No cited prior-art document discloses an isolated DNA molecule as that one disclosed in **claim 20**. Therefore, the subject-matter of **claim 20** is considered novel in the sense of Art. 54(1) and (2) EPC.
However, for the same reasons as set out under item 4-a herein above, **claim 20** does not appear inventive (Article 56 EPC).

e. The present application does not meet the requirements of Art. 54(1) and (2) EPC, because the subject-matter of **claim 22** is not new over D3.
According to the Guidelines (see G.L., C-III, 4.13), the term "comprising" has to be interpreted by the broad meaning "including", "containing" or comprehending". In view of that, and since the nucleic acid sequence listed as

SEQ ID N° 1 has 100% identity with the nucleic acid sequence listed as SEQ ID N° 1 in the present application over the 30 nucleotide length.
Moreover, the use of the term "primer", *per se*, cannot be seen as limiting the scope of the claim, contrary to the arguments of the Applicant (see letter p. 2).

Moreover, in the case wherein the reference to the aforementioned molecule would be deleted from **claim 22**, the subject-matter of **claim 22** would not be seen as novel in the sense of Art. 54(1) and (2) EPC for the same reason as set out under item 4-a herein above, because as formulated, the said **claim 22** encompasses the whole genome of maize.

f. No cited prior-art document discloses a pair of DNA molecules as that one disclosed in **claim 23**. Therefore, the subject-matter of **claim 23** is considered novel in the sense of Art. 54(1) and (2) EPC.
However, for the same reasons as set out under item 4-a herein above, and since the design of primers is a routine task of the skilled persons, **claim 23** does not appear inventive (Article 56 EPC).

g. Moreover, for the same reason as set out under item 4-a herein above, the subject-matter of **claim 24** is not new in the sense of Art. 54(1) and (2) EPC since as formulated, the said claim encompasses the whole genome of maize.

5. CONCLUDING REMARKS

a. In view of the different objections as set out under the different items herein above, no further examination is therefore possible. In the case wherein the different objections should not be overcome, the Applicant is kindly informed that the next communication will be a summon to attend oral proceedings according to Art. 116(1) EPC.

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09. Mai 2007

8 May 2007

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC *et al.*
Our Ref: N.96314 AB/eod

I refer to your Communication of 9 January 2007 and request a two month extension to the period for response, taking the total response period to six months. Please confirm in due course that this extension has been granted.

In the meantime, please acknowledge receipt of this letter by stamping and returning the attached acknowledgment copy.

Yours faithfully

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Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402

Applicant/Proprietor
Dow AgroSciences LLC, et al

Extension of time limit pursuant to Rule 84 EPC

Examination procedure

With reference to your request, the time limit for replying to the communication dated 09.01.07 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, part E-VIII, 1.6.

If no reply to the communication is received in due time, the European patent application will be deemed to be withdrawn (Article 96(3) EPC).

Examining Division

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08. Juni 2007

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7 June 2007

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC *et al.*
Our Ref: N.96314 AB/eod

This is in response to the Examination Report dated 9 January 2007. Please find attached amended claims in hand-amended and retyped formats to replace the claims currently on file. Also attached in order to bring the description into conformity with EPO practice, are the following amended pages of the description: 13, 14, 23, 44.

On behalf of the Applicants, my comments are set out below, following the Examining Division's numbering scheme.

1/2. Introduction/Amendments

No specific comments are required in response to Item 1, and it is positive that no issues arise under Article 123(2) EPC in Item 2. The amendments proposed herein are also straightforward and will I think be self-explanatory from the hand-amended claims filed herewith.

However, this is a convenient point in the discussion for some further explanation of the subject matter underlying the invention.

(i) First, please refer back to our response of 19 September 2006 for discussion of what an "event" is. As discussed there, an event is a specific integration of a transgene into a plant at a specific, initially unknown, genetic locus. Put another way, an event relates to exactly what happened in a given instance of transformation. Multiple transformations with the same construct constitute multiple events because of differences in the location, orientation, degree of fragmentation/truncation etc of the insert.

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(ii) Each event is defined by its unique "junction" sequences. A junction is the point at which two specific DNA fragments join. For example, a junction exists where insert DNA joins flanking, wild-type DNA. Thus, if the wild-type sequence were ...GGGG... and the insert sequence **CC...CC**, inserted between the third and fourth Gs, the resulting sequence would be ...GG**CC...CC**GG... and there would be two junctions, one between the second G and the first C and one between the last C and the third G.

This is further discussed in the specification at page 9, lines 21 to 25 as follows:

> *"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."*

(iii) In practice, events may well be more complicated than the notional example above. In particular, events obtained by biolistic transformation, such as the present TC1507 event, tend to be more complex than those obtained by transformation with Agrobacterium. With biolistically obtained events, there can be repeated and/or fragmented DNA on both sides of the core insert. This is important to an understanding of the present invention as follows.

(iv) Please see Table 1 at pages 30/31, and also Figure 1. These describe the genomic locus of the TC1507 event in terms of "Regions". At the 5′ end, Regions 1-3 are wild-type maize sequence and, similarly, regions 15-16 are wild-type sequence at the 3′ end. Region 9 is the PHI8999A insert itself that carries the transgene and its control sequences. However, as can clearly be seen from Figure 1, this was not a straightforward insertion of the PHI8999A insert neatly into the wild-type genome. Rather, there is a lot of broken-up, complex, repeated/fragmented sequence at either end of PHI8999A. This accounts for regions 4, 5, 6, 7 and 8 at the 5' end and regions 10, 11, 12, 13 and 14 at the 3' end. These complex flanking regions have homology to various parts of the core insert itself.

Therefore, there are not just two unique junctions involved, there are many unique junctions, i.e. one between each pair of adjacent regions shown in Table 1 and Figure 1. Identification of any of these junctions is diagnostic of the existence of the TC1507 event (as opposed to some other event or a wild-type genome).

(v) In terms of sequences:

- SEQ ID NO: 24 is the entire affected region of the genome, including Regions 1-16;
- SEQ ID NO: 25 is the core PHI8999A insert itself;
- SEQ ID NOs: 21 and 26 span the complex 5′ flanking region, and SEQ ID NO: 26 reaches a little way into the 5′ end of core insert of Region 9);
- SEQ ID NOs: 22 and 27 span the complex 3′ flanking region, with SEQ ID NO: 27 reaching a little way into the 3′ end of Region 9;
- various other sequences in the sequence listing are the sequences of the various Regions, for example SEQ ID NO: 30 is the sequence of Region 3; and

- still other sequences are the sequences of the primers that span the junctions between the various regions.

(vi) To aid in understanding of this, please find attached an annotated version of Figure 1. This shows the locations of SEQ ID NOs: 24, 25, 21/26 and 22/27. It also matches the Regions (see above regarding SEQ ID NO: 30/Region 3 for example) to their SEQ ID NOs and shows where a number of the primers bind.

3. Clarity

3(a) SEQ ID NOs: 48 and 53 appear to be somehow erroneous and have been removed from claim 9 and various other claims.

3(b) What the Examining Division says is apparently not correct. The Applicant has reviewed this in detail and can explain exactly why each of the primers mentioned in claim 24 hybridises to a junction sequence (with the exception of now-deleted SEQ ID NOs: 48/53).

As discussed in the general explanation above, there are actually many unique junctions here, not just two. Therefore, whilst it is true that SEQ ID NOs: 52 and 53 hybridise to the junctions between Regions 8/9 and 10/11 respectively, the other primers hybridise to other junctions as set out in the following table.

Primer	Hybridises at junction of Regions	Position on SID 24	Actual junction point between regions
45	3/4	1672-1691	1681-1682
46	4/5	2007-2026	2016-2017
47	5/6	2328-2347	2337-2338
49	7a/7b	2549-2568	2558-2559
50	7b/7c	2687-2706	2696-2697
51	7c/8	2702-2721	2711-2712
52	8/9	2820-2839	2829-2830
54	10/11	9556-9575	9565-9566
55	11/12	9686-9705	9693-9696*
56	12/13	10078-10097	10087-10088
57	13/14	10267-10286	10275-10286

*Note that this junction contains a few "filler" nucleotides between the regions of homology to other parts of the insert, which is an artifact of biolistic transformation. The primer still spans the junction region.

3(c) Claim 37 has been amended to be dependent on claim 10 instead of claim 36. By way of explanation, the dependency of previous claim 37 on previous claim 36 reflected the dependency of original PCT claim 64 on original PCT claim 61. However, that was clearly erroneous because, as the Examiner says, a kit claim should not be dependent on a method claim. Clearly, original PCT claim 64 should have been dependent on original PCT claim 63, in which case (taking account of the various claim rearrangements since filing), present claim 37 needs to be dependent of present claim 10.

4. Novelty/Inventive Step

4(a) From the above discussion, it will be apparent that SEQ ID NOs: 21 and 22 span the complex flanking regions at the 5′ and 3′ ends of the PHI8999A insert, and hence encompass numerous junction sequences. Therefore, they are novel for the same reasons as SEQ ID NOs: 24/26/27.

As to inventive step, please refer again to the discussion in our response of 19 September 2006. It is true that the TC1507 event discussed in the present Application is the same event that is discussed in D1. However, it is entirely wrong to say that the present invention is any kind of "alternative" to the teaching of D1. The present invention resides in the provision of means for detecting the TC1507 event. The solution to the problem of providing such detection means lay in the difficult and non-obvious process of locating and characterising TC1507 within the transformed maize genome. Once the Inventors has identified and characterised TC1507, the critical junction sequences were available to them and hence it became possible to design detection primers and devise detection methods. On this basis, it is submitted that both claims to methods and claims to primer sequences are allowable. Similarly, the longer sequences of claim 1 (SEQ ID NOs: 24, 21/26 and 22/27) can also be claimed because they are the basis for the design of the primers, their identification was non-obvious and their sequence was not known or predictable *a priori*.

4(b-d) Similar reasoning applies as for Item 4(a).

4(e) With respect, the Examining Division's interpretation of claim 22, and of citation D3, appears to be mistaken. The meaning of the term "comprising" is well understood, but the Examining Division is wrong to suggest that the term "primer" can be ignored. In line with standard EPO practice, claim 22 must be interpreted as direct towards isolated sequences suitable for use as primers. The sequence of SEQ ID NO: 1 of D3 is 1,403 nucleotides long and is plainly not suitable for such use. For example, primers are short pieces of DNA, typically 15 to 21 bases in length and are synthesised as single strands which would be technically difficult (if not impossible) for a 1403 base strand. Moreover, it is completely standard EPO practice to use the term "comprising" when defining a sequence that is novel by virtue of its isolation from within a larger sequence (see any Patent to an isolated gene or promoter, for example).

Further, it is mistaken to suggest that claims such as claim 22 can possibly be anticipated by whole genomes, even if the whole genome sequences are known. A whole genome could not conceivably be suitable for use as a primer, and nor could it be considered to be an "isolated" sequence (again, see any Patent direct to an isolated gene). Even the longer

sequences discussed in Item 4(a) above are very, very much smaller than an entire maize genome and are novel by virtue of the term "isolated".

4(f) No specific comments seem to be required; the subject matter of the Application is novel and inventive for the reasons discussed herein, and those same reasons apply to claim 23.

4(g) For the reasons discussed in 4(e) (and 4(a)) above, this claim to "isolated" sequence does not encompass the whole genome of maize.

5. Concluding Remarks

It is believed that the various objections have been overcome and the Application is now in condition for grant. If any minor points arise that the Examiner feels could easily be discussed by telephone, he is welcome to call me to talk about those. If substantive objections arise, I request that another Examination Report be issued. If the Examining Division is minded to refuse the Application without issuing another Written Communication, Oral Proceedings are requested as a precaution.

Nevertheless, it is submitted that it will very likely be inappropriate to issue a Summons to Oral Proceedings as the next Communication. It is true that this is the second Examination Report, and indeed that the EPO issued an Opinion on this Application in its capacity as ISA in the International phase. Nonetheless, there has still been no meaningful examination of the issue of inventive step. As discussed above, and in the September 2006 response, the invention here lies in the provision of means for detecting the TC1507 event in maize plants. The Examining Division has made no substantive comments at all on the reasoning provided in support of the inventive step of this subject matter. Therefore, it seems to us that it cannot be right to issue a Summons to Oral Proceedings until and unless the Applicant has the chance to deal with properly reasoned inventive step objections in writing. Aside from this, the clarity issues have surely now been resolved, and the only other point that would appear likely to lead to the issuance of a Summons to Oral Proceedings is the suggestion that the claims somehow read onto whole maize genomes. As discussed above, the Examining Division's arguments in this respect strike the Applicant as simply mistaken and in contravention of established EPO practice regarding claim interpretation in the context of novelty in sequence cases.

I look forward to hearing from the Examining Division again in due course. In the meantime, please acknowledge receipt of this letter by stamping and returning the acknowledgement copy provided.

Yours faithfully

A. BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. 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.

3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.

4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.

5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.

6. 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 or 27.

7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

8. 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.

9. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

10. 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.

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

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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

and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

31. 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: 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

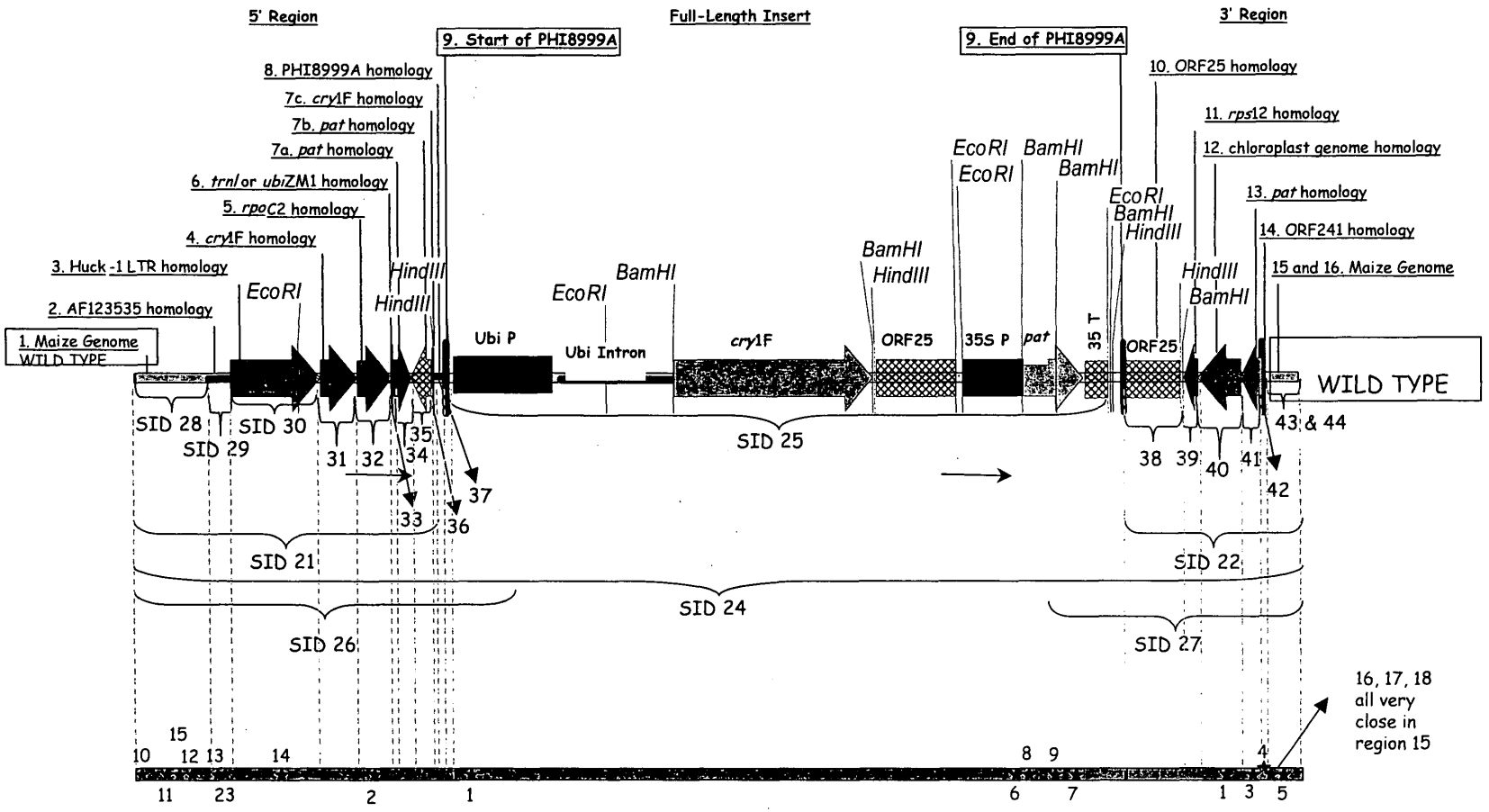
35. The method of claim 34 wherein said primer pair is selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

36. 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, 49, 50, 51, 52, 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.

37. The DNA detection kit of claim 10 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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



This diagram shows where primers are located by SEQ ID number. The green is flanking, the grey is insert. The stars denote approximate locations of primers, number according to SEQ ID number. [Note: not to scale.]

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.
2. 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.
3. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25.
4. The kit of claim 2, further comprising at least a second primer which recognizes a sequence within SEQ ID NO:22.
5. The kit of claim 3, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.
6. 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 or 27.
7. 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.
8. 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.
9. 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.
10. 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.

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

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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.

23. 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, or 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.

24. 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.

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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,

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.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, 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 descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, 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 seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, 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 descent plant's genome.

31. 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: 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.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

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

9 →
(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.

35. The method of claim 34 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.

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

9 →
(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.

37. The DNA detection kit of claim 10 ~~36~~ 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.

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

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

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

~~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.~~

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The examination is being carried out on the following application documents:

Description, Pages

1-12, 15-22, 24-43	as published			
13, 14, 23, 44	received on	08.06.2007	with letter of	07.06.2007

Sequence listings part of the description, Pages

1-21	as published

Claims, Numbers

1-38	received on	08.06.2007	with letter of	07.06.2007

Drawings, Sheets

1/1	as published

1. INTRODUCTION

When drafting this communication, the Examining Division has taken the arguments provided by the Applicant in his reply of June, 7th 2007, into consideration.

In view of the amended claims, the following document (D5-D7), cited in the ISR, is referred to in this communication; the numbering will be adhered to in the rest of the procedure:
D5: JP-A-2001136983 (2001-05-22). NAT. FOOD RES. "CORN AND METHOD

FOR DETECTING RECOMBINATION GENE FROM PROCESSED FOOD CONTAINING THE SAME".

D6: PETERSON D.G. ET AL., (May, 2002). "Integration of Cot analysis, DNA cloning, and high-throughput sequencing facilitates genome characterization and gene discovery". Genome Research Vol. 12(5), pages. 795-807. ISSN 1088-9051.

D7: WO-A-0185974. UNIV SOUTHERN CALIFORNIA [US]. "RETROVIRAL VECTORS COMPRISING AN ENHANCED 3' TRANSCRIPTION TERMINATION STRUCTURE".

2. ART. 123(2) EPC

The amendments filed with the letter dated June, 7th 2007 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. CLARITY (Art. 84 EPC)

a. Present **claim 21** reads: "*A method of detecting the presence of DNA corresponding to the TC1507 even 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".

However, in absence of any technical feature relating to the 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, present **claim 21** 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 said probes associated with the TC1507 event.
Consequently, the examination will be carried out for those parts of the subject-matter that, in view of the description, appear to be supported, namely the probes listed as SEQ ID N° 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57.

b. Present **claim 34** reads: "*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 N° 26 or SEQ ID N°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 com tissue with said primer pair;
(c) performing DNA amplification and analysing for an amplicons".

However, in absence of any technical feature relating to the sequence diagnostic for said TC1507 event insertion, present **claim 21** 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 said diagnostic sequence associated with the TC1507 event.
Consequently, the examination will be carried out for those parts of the subject-matter that, in view of the description, appear to be supported, namely the nucleotide sequences amplified in the reaction H, I, J and K (see Tab. 2 and Tab. 7).

c. Present **claim 35,** which relates to the method of claim 34 wherein the primer pair is selected from the group consisting of SEQ ID N°° 1-20, 23, 45-47, 49-52, 54-57 and complements thereof, lacks clarity in the sense of Art. 84 EPC because the aforelisted sequences correspond to primer sequences and not to primer pairs.

d. For the same reasons as set out under item 3-c herein above, present **claim 37** lacks clarity in the sense of Art. 84 EPC.

4. NOVELTY (Art. 52(1) and Art. 54(1)(2) EPC)

a. No cited prior-art document discloses an isolated DNA molecules comprising a nucleotide sequence identified as SEQ ID N° 21, 22, 24, 26 and 27. Therefore, the subject-matter of **claims 1, 3, 5, 7, 9, 10 and 37** is considered novel in the sense of Art. 54(1) and (2) EPC.

b. However, the present application does not meet the requirements of Art. 52(1) EPC, because the subject-matter of **claims 2 and 4** is not new in the sense of Art. 54(1) and (2) EPC in view of the primer n° 6 disclosed in D5 and in view of the MRCot2C09 fragment disclosed in D6.

c. Moreover, the present application does not meet the requirements of Art. 52(1) EPC, because the subject-matter of **claims 6 and 8** is not new in the sense of Art. 54(1) and (2) EPC in view of the sequence listed as SEQ ID N° 5 disclosed in D7.

d. Moreover, present **claim 11** relates to a kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID N° 24. According to the Guidelines (see G.L., C-III, 4.8), said claim must be construed as meaning merely reagent suitable for identifying event TC1507. Then, since the present claim is silent as to any technical feature characterizing the said kit, any kit of the prior art can be seen as novelty destroying in the sense of Art. 54(1) and (2) EPC.

e. No cited prior-art document discloses methods wherein the presence of maize event TC1507 in a biological sample or progeny is identified/detected. Therefore, the subject-matter of independent **claims 12, 19, 21, 25, 26, 27, 34, 36 and 38** is considered novel in the sense of Art. 54(1) and (2) EPC.

f. No cited prior-art document discloses an isolated DNA molecule comprising the amplicon produced by the method of claim 19.Therefore, the subject-matter of **claim 20** could be considered novel in the sense of Art. 54(1) and (2) EPC.

g. No cited prior-art document discloses an isolated DNA nucleotide primer as that

one disclosed in claim 22. Therefore, the subject-matter of **claim 22** could be considered novel in the sense of Art. 54(1) and (2) EPC.

h. No cited prior-art document discloses 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 N° 26 or SEQ ID N° 27, its complements thereof, to function as DNA primers or probes for diagnosing the TC1507 event. Therefore, the subject-matter of independent **claim 23** could be considered novel in the sense of Art. 54(1) and (2) EPC.

i. No cited prior-art document discloses an isolated DNA molecule as that one disclosed in **claim 24**. Therefore, the subject-matter of **claim 24** could be considered novel in the sense of Art. 54(1) and (2) EPC.

j. No cited prior-art document discloses an isolated DNA sequence as that one disclosed in **claim 31.** Therefore, the subject-matter of **claim 31** could be considered novel in the sense of Art. 54(1) and (2) EPC.

k. Present **claim 32** relates to a pair of isolated DNA sequences, each comprising at least ten nucleotides and which when used together in a DNA amplification procedure will procedure an amplicon diagnostic for event TC1507.
Since the use of said primer pairs cannot be seen as a technical feature and since the present claim is silent as to any technical feature relating to the primer sequence, any primer pair of the prior art could be seen as novelty destroying in the sense of Art. 54(1) and (2) EPC.

l. No cited prior-art document discloses the pair of isolated DNA sequences as that one disclosed in **claim 33**. Therefore, the subject-matter of independent **claim 33** is considered novel in the sense of Art. 54(1) and (2) EPC.

5. INVENTIVE STEP (Art. 52(1) and 56 EPC)

a. Present **claim 1** reads: "*An isolated DNA molecule comprising a nucleotide*

sequence identified as SEQ ID N°21, 22, 24, 26 or 27".

Present example 1 discloses the protocol of transformation of maize using particle bombardment and regeneration of transgenic plants containing the Cry1F. It appears that "*microscopic tungsten particles were coated with purified PHI8999A DNA (SEQ ID N°25) and accelerated into the cultured embryos (...). Only insert PHI8999A was used during the transformation and no additional plasmid DNA was incorporated into the transformant*" (see p. 24, l. 12-15).

In his reply dated June, 7th 2007, the Applicant provides further explanations relating to biolistic transformation. Thus, it appears that events obtained by biolistic transformation, such as the present TC1507 event, tend to be more complex than those obtained by transformation with *Agrobacterium*. With biolistic obtained events, there can be repeated and/or fragmented DNA on both sides of the core insert. Thus, as can be seen from Fig. 1, the TC1507 even is not a straightforward insertion of the PHI8999A insert (SEQ ID N°25) neatly into the wild-type genome. Rather, there is a lot of broken-up, complex, repeated/fragmented sequence at both end of PHI8999A. This accounts for **region 4** (location 1681-2016 in the SEQ ID N°24), **region 5** (location 2016-2337 in the SEQ ID N°24), **region 6** (location 2337-2354 in the SEQ ID N°24), **region 7** (location 2354-2711 in the SEQ ID N°24) and **region 8** (location 2711-2829 in the SEQ ID N°24) at the 5'-flanking region and **region 10** (location 9016-9565 in the SEQ ID N°24), **region 11** (location 9565-9693 in the SEQ ID N°24), **region 12** (location 9693-10087 in the SEQ ID N°24), **region 13** (location 10087-10275 in the SEQ ID N°24) and **region 14** (location 10275-10358 in the SEQ ID N°24) at the 3'-flanking region.

Region 1 (location 1-669 in the SEQ ID N°24), **region 2** (location 669-869 in the SEQ ID N°24) and **region 3** (location 869-1681 in the SEQ ID N°24) at the 5'-flanking region and, similarly, **region 15** (location 10358-10612 in the SEQ ID N°24) and **region 16** (location 10612-11361 in the SEQ ID N°24) at the 3'-flanking region are wild-type maize sequences.

Thus, the claimed sequences SEQ ID N°21, 22, 24, 26 and 27 are associated with the TC1507 event. Accordingly, the only underlying technical problem that can be

recognised is the provision of sequences for detecting the TC1507 event.

However, different PCR analysis are performed to determine if the 5-'flanking region is specific to the TC1507 event. It appears that reactions A, B, C and D produce amplicons (respectively location 25-324 in SEQ ID N° 24, location 25-480 in SEQ ID N° 24, location 759-1182 in SEQ ID N° 24 and location 415-1182 in SEQ ID N° 24) that are not unique to the 5-'flanking region of maize event TC1507 but are also present in the unmodified corn line (see p. 32, l. 23-25 and Tab. 2). Reactions E and F both produce amplicons (respectively location 4750-5794 in SEQ ID N° 24 and location 4827-5308 in SEQ ID N° 24) that are specific for a maize line transformed with a *pat* gene region but not to the TC1507 event (see p. 32, l. 8-9 and Tab. 2).
Only reactions H and I (respectively location 2158-3069 in SEQ ID N° 24 and location 2158-3001) both do not produce amplicon in the unmodified control corn line and are thus specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2).

Moreover, five PCR are carried out to determine if the 3-'flanking region is specific to the TC1507 event (p. 40, l. 6-10 and Tab. 7). It appears that only reactions J and K produce 342bp-amplicon and 252bp-amplicons (respectively location 10199-10541 in SEQ ID N° 24 and location 10290-10541 in SEQ ID N° 24) that are specific for the TC1507 event (see p. 40, l. 3-5 and Tab. 7).

In view of the technical effect linked to the claimed sequences, namely the detection of the TC1507 event, the Examining Division is of the opinion that the subject-matter of **claim 1** does 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.

b. Moreover, as already disclosed, reactions H, I, J and K do not produce amplicons in the unmodified control corn line and are thus specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2). In view of that, the Examining Division is of the opinion that present **claims 3 and 5**, which relates to a kit for identifying the TC1507 event, could involve an inventive step over the prior art (Art. 56 EPC) in view of the prior art.

c. Moreover, as already disclosed, reactions H, I, J and K do not produce amplicons in the unmodified control corn line and are thus specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2). The said amplicons comprise sequences which hybridizes with SEQ ID N° 21 and SEQ ID N° 25, contiguous therewith or sequences which hybridizes with SEQ ID N° 22 and SEQ ID N° 25, contiguous therewith. In view of that, the Examining Division is of the opinion that present **claim 7**, which relates to a kit containing probes hybridizing to the aforelisted amplicons, could involve an inventive step over the prior art (Art. 56 EPC) in view of the prior art.

d. Since amplicons produced by reactions H, I, J and K are specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2), only the probes hybridizing to the said amplicons would be considered specific to the TC1507 event.
Consequently, the Examining Division is of the opinion that present **claim 9** do not solve the technical problem over its entire scope since probes comprising the sequences SEQ ID N° 45, 46, 54, 55 and 56 would not detect the amplicons produced during the reactions H, I, J and K. Therefore, the subject-matter of **claim 9** 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.

e. For the same reasons as set out under item 5-j herein above, the kit of **claim 10** comprising a primer pair wherein each primer from SEQ ID N° 26 and SEQ ID N° 27 is on opposite sides of the diagnostic sequence for the TC1507 event could involve an inventive step over the prior art (Art. 56 EPC) in view of the prior art.

f. Present **claim 12** reads: "*A method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which especially recognises a sequence within SEQ ID N°21 or SEQ ID N°22*".

From the description, the said sequences SEQ ID N° 21 and 22 both correspond to the flanking regions of TC1507 (see p. 4, l. 6-7).
With respect to the sequence SEQ ID N° 21 (location 1-2829 in SEQ ID N° 24), the alignment sequence analysis indicates high homology between the region 1-1681 of the sequence SEQ ID N° 21 (location 1-1681 in the SEQ ID N° 24) and

maize genomic sequences (see example 5 and Tab. 1).
Moreover, different PCR analysis are performed to determine if the 5-'flanking region is specific to the TC1507 event. It appears that reactions A, B, C and D produce amplicons (respectively location 25-324 in SEQ ID N° 24, location 25-480 in SEQ ID N° 24, location 759-1182 in SEQ ID N° 24 and location 415-1182 in SEQ ID N° 24) that are not unique to the 5-'flanking region of maize event TC1507 but are also present in the unmodified corn line (see p. 32, l. 23-25 and Tab. 2).
Then, it appears that reaction E and F both produce amplicons (respectively location 4750-5794 in SEQ ID N° 24 and location 4827-5308 in SEQ ID N° 24) that are specific for a maize line transformed with a *pat* gene region but not to the TC1507 event (see p. 32, l. 8-9 and Tab. 2).
Moreover, it appears that only reactions H and I (respectively location 2158-3069 in SEQ ID N° 24 and location 2158-3001) both do not produce amplicon in the unmodified control corn line and are thus specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2).

With respect to the sequence SEQ ID N° 22 (location 9015-11361 in SEQ ID N° 24), the alignment sequence analysis indicates high homology with three regions of maize chloroplast DNA (p. 36, l. 18-21). Moreover, five PCR are carried out to determine if the 3-'flanking region is specific to the TC1507 event (p. 40, l. 6-10 and Tab. 7). It appears that only reactions J and K produce 342bp-amplicon and 252bp-amplicons (respectively location 10199-10541 in SEQ ID N° 24 and location 10290-10541 in SEQ ID N° 24) that are specific for the TC1507 event (see p. 40, l. 3-5 and Tab. 7).

Thus, it appears that only part of the flanking regions of SEQ ID N° 21, 22 are specific to the TC1507 event. In view of that, the Examining Division is of the opinion that present **claim 12** does not solve the technical problem over its entire scope. Therefore, the subject-matter of **claim 12** 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.

g. For the same reasons as set out under item 5-a herein above, the Examining Division is of the opinion that present **claims 13 and 15** does not solve the

technical problem over its entire scope. Therefore, the subject-matter of **claims 13 and 15** 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.

h. However, in view of the fact that reactions H, I, J and K do not produce amplicons in the unmodified control corn line and are thus specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2) wherein the amplicons are amplified with respectively the primer set SEQ ID N° 1 and 2, primer set SEQ ID N° 2 and 23, primer set SEQ ID N° 3 and 5 and primer set SEQ ID N° 4 and 5, the Examining Division is of the opinion that present **claims 14 and 16-18** could involve an inventive step over the prior art (Art. 56 EPC) in view of the prior art.

i. For the same reasons as set out under item 5-c herein above, the Examining Division is of the opinion that present **claim 19**, relating to a detection method using the aforelisted primer sets, **claim 20**, relating to the amplicons obtained with the aforelisted primer sets, could involve an inventive step over the prior art (Art. 56 EPC) in view of the prior art.

j. For the same reasons as set out under item 5-d herein above, the Examining Division is of the opinion that present **claims 21 and 36**, relating to a detection method using the aforelisted probes, do not solve the technical problem over its entire scope. Therefore, the subject-matter of **claims 21 and 36** 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.

k. For the same reasons as set out under item 5-a herein above, the Examining Division is of the opinion that present **claims 22, 23, 24 and 31-33 and 37** does not solve the technical problem over its entire scope. Therefore, the subject-matter of **claims 22, 23, 24 and 31-33 and 37** 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.

l. For the same reasons as set out under item 5-a herein above, the Examining Division is of the opinion that present **claim 25**, relating to a method for confirming

seed purity, and **claim 26**, relating to a method for screening seed for the presence of event TC1507, does not solve the technical problem over its entire scope. Therefore, the subject-matter of **claims 25-26** 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.

m. Present **claim 27** relates to a method for producing an insect resistant corn plant comprising breeding with a plant wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID N° 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57, or complements thereof and selecting progeny by analysing the presence of at least one of the said sequences.

As already disclosed under item 5-a herein above:
- reactions A, B, C and D produce amplicons (respectively location 25-324 in SEQ ID N° 24, location 25-480 in SEQ ID N° 24, location 759-1182 in SEQ ID N° 24 and location 415-1182 in SEQ ID N° 24) that are present in the unmodified corn line (see p. 32, l. 23-25 and Tab. 2);
- reaction E and F both produce amplicons (respectively location 4750-5794 in SEQ ID N° 24 and location 4827-5308 in SEQ ID N° 24) that are specific for a maize line transformed with a *pat* gene region but not to the TC1507 event (see p. 32, l. 8-9 and Tab. 2);
- H and I (respectively location 2158-3069 in SEQ ID N° 24 and location 2158-3001) in SEQ ID N° 24 do not produce amplicons in the unmodified control corn line and are thus specific to the TC1507 event (see p. 32, l. 18-20 and Tab. 2);
- J and K do not produce amplicons in the unmodified control corn line and are thus specific to the TC1507 event (see p. 40, l. 3-5 and Tab. 7);
- reactions L, M and N produce amplicons that are not unique in the 3'-flanking region of TC1507 but are also present in the unmodified control corn line (see p. 40, l. 8-10 and Tab. 7).

In view of that, it appears that only a nucleotide sequence falling within the sequence of the amplicons produced by the reactions H, I, J and K would allow the detection of the TC1507 event. Thus, only the nucleotide sequences listed as SEQ ID N° 47, 49, 50, 51, 52 and 57 can detect the TC1507 event since they hybridize

to the H-, I-, J- and K-reaction products.
Consequently, the Examining Division is of the opinion that present **claim 27**, and dependent **claims 28-30** do not solve the technical problem over its entire scope since the nucleotide sequences listed as SEQ ID N° 45, 46, 54, 55 and 56 do not fall within the sequences of the amplicons produced during the reactions H, I, J and K. Therefore, the subject-matter of **claim 27,** and dependent **claims 28-30,** do 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.

n. In the case wherein the scope of **claim 34** would be limited to the diagnostic sequences as amplified in the reactions H, I, J and K (see item 3-b herein above), for the same reasons as set out under item 5-c herein above, the Examining Division is of the opinion that present **claim 34-35**, relating to a method for detecting the presence of the TC1507 event insertion in corn tissue, could involve an inventive step over the prior art (Art. 56 EPC) in view of the prior art.

o. For the same reasons as set out under item 5-a herein above, the Examining Division is of the opinion that present **claim 38** does not solve the technical problem over its entire scope. Therefore, the subject-matter of **claim 38** 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. CONCLUDING REMARKS

a. The applicant is requested to file new claims which take account of the above comments.

b. 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).

c. The amendments would have to be filed taking into account the requirements of R. 50(1) 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.

d. 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.

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Application No.	Ref.	Date
04 751 097.9 - 2402	N.96314 AB	08.04.2008
Applicant: Dow AgroSciences LLC		

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).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

Helliot, Bertrand
Primary Examiner
for the Examining Division

Registered Letter
EPO Form 2001 12.07CSX

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Enclosure(s): 13 ✗ page/s reasons (Form 2906)
D5, D6, D7

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04751097.9 - 2402 / 1620571 18.06.08

Client Database System (CDS) - clean up.

Application Nr.: 04751097.9

Following clean up action in CDS the entries concerning the **Applicant (02)** have been amended and are now as follows:

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E.I. DU PONT DE NEMOURS AND COMPANY
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Where appropriate, the Register of European Patents will be updated to show the amended details.

For questions please contact the Client Data Registration department of the European Patent Office in Munich, telephone +49 (0)89 2399 2780.

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BY DHL COURIER

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62
31. Juli 2008

30 July 2008

Dear Sirs

European Patent Application No. 04751097.9
DOW AGROSCIENCES LLC *et al.*
Our Ref: N.96314 AB/zaw

I refer to your Communication of 8 April 2008 and request a two month extension to the period for response, taking the total response period to six months. Please confirm in due course that this extension has been granted.

In the meantime, please acknowledge receipt of this letter by stamping and returning the attached acknowledgment copy.

Yours faithfully

A BENTHAM
AUTHORISED REPRESENTATIVE

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Date
12.08.08

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402 / 1620571
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 dated 08.04.08 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, part E-VIII, 1.6.

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).

For the Examining Division



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OUR REF.: N.96314 AB/mr

DATE: 20 October 2008

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Please would you acknowledge safe and complete receipt of this transmission in legible form by signing and returning this sheet to us by fax

SIGNED: _____ DATE: _____

Received at the EPO on Oct 20, 2008 16:36:38. Page 20 of 38

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BY FAX & DHL COURIER

The European Patent Office
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20 October 2008

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC, *et al*
Our Ref: N.96314 AB/mr

This is in response to the Examination Report dated 8 April 2008. Please find attached new claims in retyped and hand-amended formats to replace the current claims.

AMENDMENTS

- Claim 2 has been combined with claim 3 to form new claim 2(a), and with claim 4 to form new claim 2(b).
- Claim 5 has been split into new claims 3 to 6, corresponding to claims 11 to 14 as filed.
- Claim 7 has been split into new claims 8 and 9.
- Claims 8 and 11 have been deleted.
- Consequent revisions to the dependency of various claims have therefore been made.
- Some of the sequences have been removed from claims 22 and 31.

It is proposed to defer amendment to the description until an allowable set of claims is agreed upon.

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REMARKS

It is positive that the Examining Division is able to accept that some of the claims contain inventive subject matter. However, it is submitted that others of the claims also contain inventive subject matter and that the amended set of claims filed herewith is in conformity with the requirements of the EPC.

In more detail, the Applicant's comments are set below, following the Examining Division's numbering scheme.

1. Introduction

No comments required.

2. Article 123(2) EPC

No comments required but see above regarding amendments.

3. Clarity

a. With respect, it is submitted that it is clearly not a "research program" or undue burden to identify probes (or primers) associated with the TC1507 event but rather a routine piece of scientific work. The present application provides the sequence of TC1507 and explains its structure and where the junction sequences are to be found within it. Based on this information, it is well within the capacity of the person skilled in the art to design probes (or primers) suitable for uniquely identifying TC1507. Designing such probes (or primers) is a simple matter of looking at the sequence by eye or with readily available software tools and designing a probe (or primers) corresponding to the relevant portion(s) of the sequence in terms of base pairing. Having designed the probe (or primers) in this straightforward manner, it is then a simple matter to synthesise it (them) as well. For this reason, it is submitted that claim 21 suffers from no lack of support under Article 84 EPC. If the Applicant is forced to confine its claims to particular sequences when others could just as easily be designed by the skilled person, third parties will be in a position unfairly to benefit from the Applicant's contribution to the art without falling within the terms of the claims.

b. See (a) above.

c. Claim 35 is dependent on claim 34. Claim 34 makes it clear that each member of the primer pair must be taken from within SEQ ID No. 26 or 27, that the two members of the pair must be on opposite sides of a sequence diagnostic for TC1507 (ie. a junction) and that the overall method must be a method of detecting the presence of TC1507, ie. a method that is

effective. All of these features of independent claim 34 are inherent in dependent claim 35 as well by virtue of the dependency and claim 35 cannot by definition mean anything that is at variance with independent claim 34. Seen in this light, it is clear that claim 35 means that both sequences of the pair of claim 34 are chosen appropriately from within the list contained in claim 35.

d. See (c) above.

4. Novelty

a. No comments required.

b. See the section headed "Amendments" above for discussion of the amendments to claims 2 to 5. Part (a) of new claim 2 represents a combination of previous claims 2 and 3. No objection was raised to previous claim 3 so it is submitted that new claim 2(a) is novel. It is also submitted that the same applies to new claim 2(b). The disclosure of D5/D6 does not appear to encompass combinations of first and second primers as required in claim 2(b).

c. Claim 8 has been cancelled. However, it is submitted that claim 6 (new claim 7) is novel because it requires that the detection kit be specific for junction DNA whereas the Examining Division has not alleged that the sequences of the prior art correspond to junction sequence.

d. Claim 11 has been cancelled.

e - j. No comments required.

k. There does not in fact appear to be any difficulty with the language of claim 32. As discussed above, the skilled person can use the sequences provided by the invention to design suitable probes and primers. The Application makes available to the skilled person the full range of probes and primers that can be used to identify TC1507. Claim 32 clearly refers to primer pairs that are within the skilled person's grasp. It is not the use of the primers *per se* that is the technical feature central to the claim, it is the sequence of TC1507. However, the term "when used together ... will produce and amplicon diagnostic for ..." acts to make sure that all of the claimed sequence pairs must be capable of amplifying TC1507. This in turn controls the sequence of those primer pairs by reference to the sequence of TC1507, provided in the application. Therefore, there does not appear to be any justification for the very broad reading of claim 32 suggested by the Examining Division, and hence there is no lack of novelty.

l. No comments required.

5. Inventive Step

a. Whilst the Examining Division's analysis of the technical data contained in the specification may be correct, the legal conclusions drawn from it do not appear to be correct. Claim 1 sets out the sequences of flanking regions SEQ ID Nos. 21/22 and 26/27, plus the entire TC1507 sequence of SEQ ID No. 24. These sequences all include unique patterns of junction sequences that define the TC1507 event and distinguish it both from wild-type genomic sequences and from other transgenic events. The skilled person is therefore given the means to design the full range of probes/primers that could be used to identify/amplify the TC1507 event. True, SEQ ID Nos. 21/22/24/26/27 do encompass some wild-type sequence at the extreme by 5' and 3' ends, which are presumably what gives rise to the non-TC1507-specific amplicons. However, this does not mean that SEQ ID Nos. 21/22/24/26/27 are not in compliance with Article 56 EPC. They all contain the decisive, unique junction regions and can all be used to design suitable probes/primers. The sequence disclosure of the specification also enables the skilled person to determine which probes/primers corresponding to SEQ ID Nos. 21/22/24/26/27 will be effective and which will not (ie. pairs of primers recognising only wild-type sequence). In this regard, note also that even a primer binding to the wild-type sequence can be diagnostic of TC1507 if combined with another primer from within the TC1507-unique regions.

For this reason, the Examining Division's discussion of the amplicons does not seem to be relevant to the question of inventive step.

b/c. No comments required.

d. Once again, the focus on the amplicons appears to be misleading. With reference to the table at page 3 of the Response of 7 June 2007, all of the sequences of claim 9 (new claim 10) are TC1507-unique sequences in that they hybridise at junction regions and clearly can be used to detect TC1507.

e. No comments required.

f. Once again, this discussion does not appear to be relevant to the question of Article 56 EPC. Claim 12 requires that TC1507 be identified. To do this, a sequence within SEQ ID No. 21 or 22 is used. It is true that SEQ ID Nos. 21 and 22 include some wild-type sequence but the skilled person is able to distinguish this from the TC1507-unique sequence in SEQ ID Nos. 21/22 because of the information provided in the specification and the requirement that TC1507 be identified rules out detecting the non-TC1507-specific wild type sequence out the claims.

g-i. These claims dependent upon claim 12 and the same points apply to them as in (f) above.

j. Regarding claim 21, see (f) above. Regarding claim 36, similar arguments apply but note also that (see the table at page 3 of the 7 June 2007 Response) all of the sequences in claim 36 are TC1507-unique junction sequences.

k. In claims 22 and 31, the sequences that do not hybridise at junction sequences have been removed. Claim 24 also relates only to sequences hybridising at junction points. Regarding claim 23, see the discussion in 3(a) and 5(a) above: the skilled person knows from the content of the specification how to put this claim into practice by using the TC1507-unique sequences within SEQ ID Nos. 26/27 and avoiding the wild-type sequence. Similar arguments apply to claims 32 and 33. Regarding claim 37, this is dependent on claim 11 (previously claim 10). Claim 11 makes it clear that the two primers must hybridise on opposite sides of a TC1507-diagnostic (i.e. junction) sequence and the list of sequences in claim 37 has to be read with that restriction in mind.

l. Once again, similar points apply. Claims 25 and 26 require the identification of TC1507, which rules out binding to wild-type DNA alone.

m. In claim 27, all the claimed sequences are junction-specific sequences (see the table at page 3 of the Response of 7 June 2007) so the objection to claims 27-30 does not appear to be well-founded.

n. As already discussed, claims such as claim 34 are in fact limited to TC1507-diagnostic sequences (see 5(k) above regarding claim 11, previous claim 10).

o. Once again, see the discussion above: claim 38 is limited to a method for identifying TC1507, which rules out methods that do not identify TC1507; and the skilled person is put in a position via the sequence disclosure of the application to distinguish suitable methods from unsuitable ones and design probes/primers to carry out the suitable methods.

6. Concluding Remarks

a/b. Please see attached.

c. No comments required but see the section headed "Amendments " above.

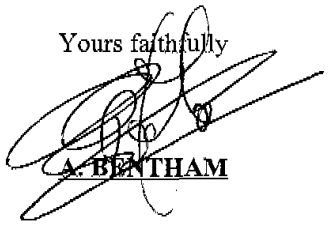
d. It is submitted that the claims comply with Rule 43(2) EPC in that the product claims represent a plurality of interrelated products, the method claims represent different but related uses of these products and that, in some cases, the different method claims represent alternative solutions to a problem that cannot readily be covered by a single claim. Nevertheless, if the Examining Division can confirm that the claims are fundamentally allowable in other, more substantive respects, the Applicant is of course willing to reexamine how many independent claims are required in response to any specific, reasoned

comments that the Examining Division may have regarding redundancy of particular independent claims.

It is believed that the various objections have been overcome and that the application is now in condition for grant (save possibly for conforming amendments to the description). If any minor points arise that the Examiner feels could easily be discussed by telephone, he is welcome to call me about those. If substantive objections remain, I request that another Examining 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.

I look forward to hearing from the Examining Division again in due course. In the meantime, please acknowledge receipt of this letter by stamping and returning the acknowledgement copy provided.

Yours faithfully



A. BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. 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, and further comprising at least:

   (a) a second primer which recognizes a sequence within SEQ ID NO:25, or

   (b) a second primer which recognizes a sequence within SEQ ID NO:22.

3. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. 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 or 27.

8. 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.

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9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

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17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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

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

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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

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and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

31. 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: 19, 20, 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

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

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(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.

35. The method of claim 34 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

36. 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, 49, 50, 51, 52, 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.

37. The DNA detection kit of claim 11 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

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CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. 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, and

~~3. The kit of claim 2,~~ further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25, or

~~4. The kit of claim 2, further comprising at least~~ a second primer which recognizes a sequence within SEQ ID NO:22.

~~X~~ 3 The kit of claim ~~2~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively, the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively, 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.

~~6.~~ 7 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 or ~~27.~~

~~8~~ ~~X~~ 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, or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

~~8. 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~~

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~~NO:27 that functions as a DNA primer or probe specific for maize event TC1507 and its progeny~~.

10 ~~9~~. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11 ~~10~~. 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.

~~11. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.~~

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

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16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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

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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.

22. 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, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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

and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

31. 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: ~~2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18~~ 19, 20~~ 23~~ 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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.

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33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

35. The method of claim 34 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

36. 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, 49, 50, 51, 52, 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.

37. The DNA detection kit of claim ~~36~~ 11 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

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20 October 2008

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC, *et al*
Our Ref: N.96314 AB/mr

This is in response to the Examination Report dated 8 April 2008. Please find attached new claims in retyped and hand-amended formats to replace the current claims.

AMENDMENTS

- Claim 2 has been combined with claim 3 to form new claim 2(a), and with claim 4 to form new claim 2(b).
- Claim 5 has been split into new claims 3 to 6, corresponding to claims 11 to 14 as filed.
- Claim 7 has been split into new claims 8 and 9.
- Claims 8 and 11 have been deleted.
- Consequent revisions to the dependency of various claims have therefore been made.
- Some of the sequences have been removed from claims 22 and 31.

It is proposed to defer amendment to the description until an allowable set of claims is agreed upon.

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REMARKS

It is positive that the Examining Division is able to accept that some of the claims contain inventive subject matter. However, it is submitted that others of the claims also contain inventive subject matter and that the amended set of claims filed herewith is in conformity with the requirements of the EPC.

In more detail, the Applicant's comments are set below, following the Examining Division's numbering scheme.

1. Introduction

No comments required.

2. Article 123(2) EPC

No comments required but see above regarding amendments.

3. Clarity

a. With respect, it is submitted that it is clearly not a "research program" or undue burden to identify probes (or primers) associated with the TC1507 event but rather a routine piece of scientific work. The present application provides the sequence of TC1507 and explains its structure and where the junction sequences are to be found within it. Based on this information, it is well within the capacity of the person skilled in the art to design probes (or primers) suitable for uniquely identifying TC1507. Designing such probes (or primers) is a simple matter of looking at the sequence by eye or with readily available software tools and designing a probe (or primers) corresponding to the relevant portion(s) of the sequence in terms of base pairing. Having designed the probe (or primers) in this straightforward manner, it is then a simple matter to synthesise it (them) as well. For this reason, it is submitted that claim 21 suffers from no lack of support under Article 84 EPC. If the Applicant is forced to confine its claims to particular sequences when others could just as easily be designed by the skilled person, third parties will be in a position unfairly to benefit from the Applicant's contribution to the art without falling within the terms of the claims.

b. See (a) above.

c. Claim 35 is dependent on claim 34. Claim 34 makes it clear that each member of the primer pair must be taken from within SEQ ID No. 26 or 27, that the two members of the pair must be on opposite sides of a sequence diagnostic for TC1507 (ie. a junction) and that the overall method must be a method of detecting the presence of TC1507, ie. a method that is

effective. All of these features of independent claim 34 are inherent in dependent claim 35 as well by virtue of the dependency and claim 35 cannot by definition mean anything that is at variance with independent claim 34. Seen in this light, it is clear that claim 35 means that both sequences of the pair of claim 34 are chosen appropriately from within the list contained in claim 35.

d. See (c) above.

4. Novelty

a. No comments required.

b. See the section headed "Amendments" above for discussion of the amendments to claims 2 to 5. Part (a) of new claim 2 represents a combination of previous claims 2 and 3. No objection was raised to previous claim 3 so it is submitted that new claim 2(a) is novel. It is also submitted that the same applies to new claim 2(b). The disclosure of D5/D6 does not appear to encompass combinations of first and second primers as required in claim 2(b).

c. Claim 8 has been cancelled. However, it is submitted that claim 6 (new claim 7) is novel because it requires that the detection kit be specific for junction DNA whereas the Examining Division has not alleged that the sequences of the prior art correspond to junction sequence.

d. Claim 11 has been cancelled.

e - j. No comments required.

k. There does not in fact appear to be any difficulty with the language of claim 32. As discussed above, the skilled person can use the sequences provided by the invention to design suitable probes and primers. The Application makes available to the skilled person the full range of probes and primers that can be used to identify TC1507. Claim 32 clearly refers to primer pairs that are within the skilled person's grasp. It is not the use of the primers *per se* that is the technical feature central to the claim, it is the sequence of TC1507. However, the term "when used together ... will produce and amplicon diagnostic for ..." acts to make sure that all of the claimed sequence pairs must be capable of amplifying TC1507. This in turn controls the sequence of those primer pairs by reference to the sequence of TC1507, provided in the application. Therefore, there does not appear to be any justification for the very broad reading of claim 32 suggested by the Examining Division, and hence there is no lack of novelty.

l. No comments required.

5. Inventive Step

a. Whilst the Examining Division's analysis of the technical data contained in the specification may be correct, the legal conclusions drawn from it do not appear to be correct. Claim 1 sets out the sequences of flanking regions SEQ ID Nos. 21/22 and 26/27, plus the entire TC1507 sequence of SEQ ID No. 24. These sequences all include unique patterns of junction sequences that define the TC1507 event and distinguish it both from wild-type genomic sequences and from other transgenic events. The skilled person is therefore given the means to design the full range of probes/primers that could be used to identify/amplify the TC1507 event. True, SEQ ID Nos. 21/22/24/26/27 do encompass some wild-type sequence at the extreme by 5' and 3' ends, which are presumably what gives rise to the non-TC1507-specific amplicons. However, this does not mean that SEQ ID Nos. 21/22/24/26/27 are not in compliance with Article 56 EPC. They all contain the decisive, unique junction regions and can all be used to design suitable probes/primers. The sequence disclosure of the specification also enables the skilled person to determine which probes/primers corresponding to SEQ ID Nos. 21/22/24/26/27 will be effective and which will not (ie. pairs of primers recognising only wild-type sequence). In this regard, note also that even a primer binding to the wild-type sequence can be diagnostic of TC1507 if combined with another primer from within the TC1507-unique regions.

For this reason, the Examining Division's discussion of the amplicons does not seem to be relevant to the question of inventive step.

b/c. No comments required.

d. Once again, the focus on the amplicons appears to be misleading. With reference to the table at page 3 of the Response of 7 June 2007, all of the sequences of claim 9 (new claim 10) are TC1507-unique sequences in that they hybridise at junction regions and clearly can be used to detect TC1507.

e. No comments required.

f. Once again, this discussion does not appear to be relevant to the question of Article 56 EPC. Claim 12 requires that TC1507 be identified. To do this, a sequence within SEQ ID No. 21 or 22 is used. It is true that SEQ ID Nos. 21 and 22 include some wild-type sequence but the skilled person is able to distinguish this from the TC1507-unique sequence in SEQ ID Nos. 21/22 because of the information provided in the specification and the requirement that TC1507 be identified rules out detecting the non-TC1507-specific wild type sequence out the claims.

g-i. These claims dependent upon claim 12 and the same points apply to them as in (f) above.

j. Regarding claim 21, see (f) above. Regarding claim 36, similar arguments apply but note also that (see the table at page 3 of the 7 June 2007 Response) all of the sequences in claim 36 are TC1507-unique junction sequences.

k. In claims 22 and 31, the sequences that do not hybridise at junction sequences have been removed. Claim 24 also relates only to sequences hybridising at junction points. Regarding claim 23, see the discussion in 3(a) and 5(a) above: the skilled person knows from the content of the specification how to put this claim into practice by using the TC1507-unique sequences within SEQ ID Nos. 26/27 and avoiding the wild-type sequence. Similar arguments apply to claims 32 and 33. Regarding claim 37, this is dependent on claim 11 (previously claim 10). Claim 11 makes it clear that the two primers must hybridise on opposite sides of a TC1507-diagnostic (i.e. junction) sequence and the list of sequences in claim 37 has to be read with that restriction in mind.

l. Once again, similar points apply. Claims 25 and 26 require the identification of TC1507, which rules out binding to wild-type DNA alone.

m. In claim 27, all the claimed sequences are junction-specific sequences (see the table at page 3 of the Response of 7 June 2007) so the objection to claims 27-30 does not appear to be well-founded.

n. As already discussed, claims such as claim 34 are in fact limited to TC1507-diagnostic sequences (see 5(k) above regarding claim 11, previous claim 10).

o. Once again, see the discussion above: claim 38 is limited to a method for identifying TC1507, which rules out methods that do not identify TC1507; and the skilled person is put in a position via the sequence disclosure of the application to distinguish suitable methods from unsuitable ones and design probes/primers to carry out the suitable methods.

6. Concluding Remarks

a/b. Please see attached.

c. No comments required but see the section headed "Amendments " above.

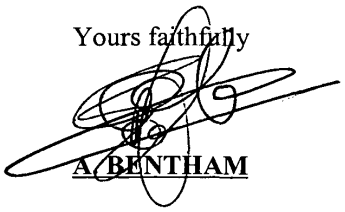
d. It is submitted that the claims comply with Rule 43(2) EPC in that the product claims represent a plurality of interrelated products, the method claims represent different but related uses of these products and that, in some cases, the different method claims represent alternative solutions to a problem that cannot readily be covered by a single claim. Nevertheless, if the Examining Division can confirm that the claims are fundamentally allowable in other, more substantive respects, the Applicant is of course willing to reexamine how many independent claims are required in response to any specific, reasoned

comments that the Examining Division may have regarding redundancy of particular independent claims.

It is believed that the various objections have been overcome and that the application is now in condition for grant (save possibly for conforming amendments to the description). If any minor points arise that the Examiner feels could easily be discussed by telephone, he is welcome to call me about those. If substantive objections remain, I request that another Examining 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.

I look forward to hearing from the Examining Division again in due course. In the meantime, please acknowledge receipt of this letter by stamping and returning the acknowledgement copy provided.

Yours faithfully



A BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. 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, and further comprising at least:

   (a) a second primer which recognizes a sequence within SEQ ID NO:25, or

   (b) a second primer which recognizes a sequence within SEQ ID NO:22.

3. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. 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 or 27.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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

and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

31. 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: 19, 20, 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

35. The method of claim 34 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

36. 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, 49, 50, 51, 52, 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.

37. The DNA detection kit of claim 11 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. 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, and

~~3. The kit of claim 2,~~ further comprising at least a second primer which recognizes a sequence within SEQ ID NO:25, or

~~4. The kit of claim 2, further comprising at least~~ a second primer which recognizes a sequence within SEQ ID NO:22.

~~3~~ The kit of claim ~~1~~, wherein said at least first and second primers comprise: the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively; the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively; 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.

~~6~~ 7 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 or 27.

~~8~~ 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; or a specific probe comprising a sequence which hybridizes with SEQ ID NO:22 and SEQ ID NO:25, contiguous therewith.

~~8. 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~~.

10 ~~9~~. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11 ~~10~~. 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.

~~11. A kit for identifying event TC1507 in a biological sample which detects TC1507 specific region within SEQ ID NO: 24.~~

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. 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, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. A method of producing an insect resistant corn plant comprising breeding with a plant,

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

and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the seed's genome.

30. A method of claim 28 further comprising obtaining seed from said descent plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.

31. 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: ~~2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18,~~ 19, 20~~, 23~~ 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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.

33. The pair of isolated DNA sequences of claim 32 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

34. 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.

35. The method of claim 34 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

36. 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, 49, 50, 51, 52, 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.

37. The DNA detection kit of claim 11 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

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Application No.	Ref.	Date
04 751 097.9 - 2402	N.96314 AB	15.06.2010
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).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

Helliot, Bertrand
Primary Examiner
For the Examining Division

Enclosure(s): 5 page/s reasons (Form 2906)

The examination is being carried out on the following application documents

Description, Pages

1-12, 15-22, 24-43	as published			
13, 14, 23, 44	received on	08-06-2007	with letter of	07.06.2007

Sequence listings part of the description, Pages

1-21	as published			

Claims, Numbers

1-38	received on	21-10-2008	with letter of	20-08-2008

Drawings, Sheets

1/1	as published			

1 INTRODUCTION

When drafting this communication, the Examining Division has taken the arguments provided by the Applicant in his reply of August, 20th 2008, into consideration.

2 ART. 123(2) EPC

The amendments filed with the letter dated August, 20th 2008 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 CLARITY (Art. 84 EPC)

3.1 Present **claim 7** relates to a DNA detection kit specific for junction DNA of maize event TC1507 and its progeny.

However, present **claim 7** lacks clarity because a kit, per se, can not be specific to a DNA molecule. The objection could be overcome with the following amendment:

A DNA detection kit comprising at least one DNA molecule that is homologuous or complementary to SEQ ID N°26 or 27 and wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of sufficient length of contiguous DNA polynucleotide to function in a DNA detection method. P

4 NOVELTY (Art. 52(1) and Art. 54(1)(2) EPC)

4.1 No cited prior-art document discloses an isolated DNA molecules comprising a nucleotide sequence identified as SEQ ID N°21, 22, 24, 26 and 27. Therefore, the subject-matter of **claim 1** is considered novel in the sense of Art. 54(1) and (2) EPC.

4.2 The same applies to **claims 2-6**.

4.3 In the case the clarity objection as set out under item 3.1 herein above would be overcome, the subject-matter of **claim 7** could be considered novel (Art. 54 (1) and (2) EPC).

4.4 Novelty of **claims 8-9** (previous claims 7), **claim 10** (previous claim 9), **claims 11 and 37** (previous claims 10 and 37), **claims 12-18** (previous claim 12-18), **claim 19** (previous claim 19) and **claim 20** (previous claim 20), **claim 21** (previous claim 21), **claim 22** (previous claim 22), **claim 23** (previous claim 23), **claim 24** (previous claim 24), **claim 25** (previous claim 25), **claim 26** (previous claim 26), claims 27-30 (previous claims 27-30), **claim 31** (previous claim 31), **claims 32-33** (previous claims 32-33), **claims 34-35** (previous claims 34-35), **claim 36** (previous claim 36) and **claim 38** (previous claim 38) has been already acknowledged in the previous communication (Art. 54(1) and (2) EPC).

5 INVENTIVE STEP (Art. 52(1) and 56 EPC)

5.1 An inventive merit has been already acknowledged for present **claim 1** in the previous communication.

5.2 Present **claim 2** relates to a kit for identifying event TC1507 in a biological sample. Sait kit comprises at least a first primer which recognises a sequence within SEQ ID N° 21 or 22 and a second primer which recognises a sequence within SEQ ID N° 25 or 22.

As the said claim comprises a number of different options, it is necessary to establish whether or not all options generate a technical effect that each would go beyond those known from the prior art.

In example 9, a PCR analysis is 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-11 control corn line revealed that Region 15 was indeed present in corn genomic DNA (see p. 39-40).

Thus, 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-11 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.

However, the region 15 corresponds to the DNA molecule listed as SEQ ID N° 43 which is part of the molecule listed as SEQ ID N° 22. In the case wherein the first primer and the second primer are both selected from the sequence SEQ ID N° 22, and more specifically in the sequence 43 (region 15), the Examining Division is, therefore, of the opinion that the present **claim 2** does not solve the technical problem over its entire scope (see Tab. 7 and 8). Thus, the subject-matter of **claim 2** 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.

5.3 The Applicant is informed that an inventive step could possibly be acknowledged for the kit of **claims 3-6** (see Tab. 3 and 8).

5.4 In the case the clarity objection as set out under item 3.1 herein above would be overcome, an inventive step could possibly be acknowledged for the kit of **claim 7.**

5.5 An inventive merit has been already acknowledged for present **claims 8-9** (previous claims 7), **claim 10** (previous claim 9), **claims 11 and 37** (previous claims 10 and 37), **claim 19** (previous claim 19), **claim 20** (previous claim 20), **claim 22** (previous claim 22), **claim 23** (previous claim 23), **claim 24** (previous claim 24), **claims 32-33** (previous claims 32-33), **claims 34-35** (previous claims 34-35), **claim 36** (previous claim 36) and **claim 38** (previous claim 38) in the previous communication.

5.6 Moreover, in view of the argument provided by the Applicant, the Examining Division is of the opinion that the subject-matter of **claims 12-18, 21** involves an inventive step over the prior art (Art. 52(1) and 56 EPC).

5.7 Present **claim 27** relates to a method for producing an insect resistant corn plant comprising breeding with a plant wherein DNA having at least one sequence selected from a group consisting of SEQ ID N° 45-57 and complement thereof forms part of the plant's genome and selecting progeny by analysing the said sequence.

However, the aforesaid sequences hybridize to junction between the different regions 3-4, 4-5, 5-6, 6-7a, 7a-7b, 7b-7c, 7c-8, 8-9, 9-10, 10-11, 11-12, 12-13 and 13-14. The said junctions do not encompass the whole PHI8999A insert which causes the plant protection.

Thus, as it is not establish that a insect resistant plant plant can be produced by breeding with a plant wherein the plant's genome comprises only one nucleotide sequence hybridizing to the aforesaid junction, the Examining Division is, therefore, of the opinion that the present **claim 27** does not solve the technical problem over its entire scope. Thus, the subject-matter of **claim 27** 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.

5.8 Dependent **claims 28-30** appear not to contain any technical features that would, in combination with the features of claim 27 overcome the objection to lack of inventive step (Art. 56 EPC) as the features referred in the said claim fall into the range of the conventional nature and routine for the skilled person in view of the general knowledge at the date of the claimed priority.

5.9 Present **claim 31** relates to an isolated DNA sequence.

The sequences 19 and 20 are use for amplifying the cry1F gene. Since the cry1F is a well known gene (see D1) and in absence of any technical effect linked to the choice of the sequences 19 and 20, the technical problem to be solved by the subject-matter of said **claim 31** may, thus, be regarded as the provision of alternative DNA sequences.

The solution provided in **claim 31**, namely the sequences 19 and 20, cannot be considered as complying with Art. 56 EPC for inventive step, as the choice of this particular sequences appears to be a random selection among the many sequences that can be derived from the gene cry1F. Therefore, the subject-matter of **claim 31** does not involve an inventive step (Art. 52(1) and 56 EPC).

6 CONCLUDING REMARKS

In view of the different objections as set out under the different items herein above,no further examination is therefore possible.In the case wherein the different objections should not be overcome, the Applicant is kindly informed that the next communication will be a summon to attend oral proceedings according to Art. 116(1) EPC.

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FAX TRANSMISSION FORM

OUR REF.: N.96314 AB/mr

DATE: 7 October 2010

YOUR REF.: 04751097.9-2402

NO. OF PAGES: 2
(INCLUDING THIS PAGE)

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80335 Munich
Germany

7 October 2010

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC., *et al*
Our Ref: N.96314 AB/mr

I refer to your Communication of 15 June 2010 and request a two month extension to the period for response, taking the total response period to six months. Please confirm in due course that this extension has been granted.

In the meantime, please acknowledge receipt of this letter by stamping and returning the attached acknowledgment copy.

Yours faithfully

Pamela Tuxworth

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Received at the EPO on Oct 07, 2010 16:41:37. Page 2 of 2

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Our Ref: N.96314 AB/mr

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GRANDE BRETAGNE

Date
13.10.10

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402 / 1620571
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 15.06.10 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, part 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).

For the Examining Division

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FAX TRANSMISSION FORM

OUR REF.: N.96314 AB/mr

DATE: 16 December 2010

YOUR REF.: 04751097.9-2402

NO. OF PAGES: 2
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16 December 2010

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC., *et al*
Our Ref: N.96314 AB/mr

I refer to the Examination Report dated 15 June 2010 and to your Communication of 13 October 2010 granting a two-month extension of time. This letter is to request a further extension of time of either one or two months. I regret that we have not been able to obtain instructions from our clients, I think partly in light of a change of responsibility amongst the Applicant's in house patent staff and external US Attorneys, and I am doubtful that we will be able to respond by the current deadline in view of the holiday season as well the need for communication between the three Applicants (two of whom are in one group of companies, but the third is separate). Therefore, a further extension of time would be much appreciated.

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Yours faithfully

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16 December 2010

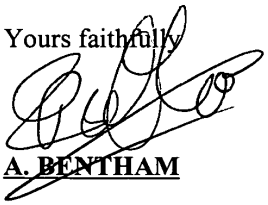
Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC., *et al*
Our Ref: N.96314 AB/mr

I refer to the Examination Report dated 15 June 2010 and to your Communication of 13 October 2010 granting a two-month extension of time. This letter is to request a further extension of time of either one or two months. I regret that we have not been able to obtain instructions from our clients, I think partly in light of a change of responsibility amongst the Applicant's in house patent staff and external US Attorneys, and I am doubtful that we will be able to respond by the current deadline in view of the holiday season as well the need for communication between the three Applicants (two of whom are in one group of companies, but the third is separate). Therefore, a further extension of time would be much appreciated.

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Date: 23-12-2010

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402 / 1620571

Applicant/Proprietor: Dow AgroSciences LLC, et al

Rejection of request for extension of time limit issued pursuant to Rule 132 EPC

Your request dated 16/12/2010 for an extension of the time limit has been refused for the reason indicated below:

☐ Your request was received by the European Patent Office on _____ , i.e. after expiry of the time limit which ended on _____ .

☒ The reasons given in the request are not sufficient, see Guidelines EPO, Part E-VIII, 1.6.

☐ The time limit in question is not a time limit set by the European Patent Office but a time limit laid down by the European Patent Convention. It cannot therefore be extended.

☐ The time limit set in a communication under Rule 71(3) EPC is not extendable.

A separate communication will be issued concerning the legal consequence that will ensue.

For the Examining Division

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Date
02-02-2011

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402 / 1620571
Applicant/Proprietor	
Dow AgroSciences LLC, et al	

Noting of loss of rights pursuant to Rule 112(1) EPC

The European patent application is deemed to be withdrawn under Article 94(4) EPC, because the invitation to file observations on the communication from the Examining Division dated 15.06.10 was not complied with.

Means of redress

Request for a decision (R. 112(2) EPC)
If the applicant considers that the finding of the European Patent Office is inaccurate, he may, within a (non-extendable) period of **two months** after notification of this communication, apply in writing for a decision on the matter. The application can only lead to the finding being reversed if this does not actually correspond to the factual or legal situation.

Further processing (Art. 121 EPC)
The legal consequence of the failure to observe the time limit shall be deemed not to have ensued if, within a (non-extendable) period of **two months** after notification of this communication, further processing is requested by payment of the fee prescribed under Article 2(12) of the Rules relating to Fees and the omitted act is completed (R. 135(1) EPC).

Important note to users of the automatic debiting procedure
The fee for further processing will be debited automatically on the day on which the above-mentioned omitted act is completed (see Arrangements for the automatic debiting procedure, Supplement to OJ EPO 3/2009).

For the Examining Division

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Registered letter
EPO Form 2021A 12.07 (24/01/11) ADWI 11

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Letter accompanying subsequently filed items

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The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	04751097.9
Applicant's or representative's reference	N.96314 AB

	Description of document	Original file name	Assigned file name
1	Request for further processing	N96314 EP AB mr Response with FP - Feb 11.pdf	RFPR-1.pdf
2	Amended claims	N96314 EP AB mr Amended claims - Feb-11.pdf	CLMSPAMD-1.pdf
3	Amended claims	N96314 EP AB mr [Tracking] Amended claims - Feb-11.pdf	CLMSPAMD-2.pdf
4	Amended description	N96314 EP AB mr Amended pages 4, 5, 6 and 7 - Feb 11.pdf	DESCPAMD-1.pdf
5	Amended description	N96314 EP AB mr hand amended pgs 4-7.pdf	DESCPAMD-2.pdf

	Fees	Factor applied	Fee schedule	Amount to be paid
15-1	122 Fee for further processing (non fee related cases)	1	225.00	225.00
	Total:		**EUR**	**225.00**

	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:	28300297
	Account holder:	Pioneer Hi-Bred International, Inc.

Signatures

Place:

Date: **22 February 2011**

Signed by:	GB, J A Kemp & Co., A. Bentham 23464
Capacity:	(Representative)

J.A.KEMP & CO.

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22 February 2011

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES LLC., *et al*
Our Ref: N.96314 AB/mr

With reference to the Communication dated 2 February 2011, I request further processing of this Application. Please debit the further processing fee (EUR 225) from deposit account no. 2830.0297 in the name of Pioneer Hi-Bred International, Inc., failing which from J A Kemp & Co's deposit account no. 2805.0038.

To complete the omitted act, namely a response to the Communication dated 15 June 2010, the following amendments and comments are offered.

AMENDMENTS

Please find attached new claims in "tracking" and "clean" formats.

- Claim 2 has been amended to remove the possibility that both primers hybridise to SEQ ID NO: 22.

- As requested by the Examining Division, claim 7 has been amended to clarity its language without changing its meaning.

- Claims 27 to 30 have been deleted and the remaining claims renumbered and reappended accordingly.

- Reference to SEQ ID NOS: 19 and 20 has been removed from claim 31 (new claim 27).

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Regarding the description, please find attached replacement pages 4-7 and 7a-7e. The "Summary of Invention" section has been conformed to the claims and claims counterpart language has been added. Hand-amended versions of pages 4-7 and 7e also attached for reference.

COMMENTS ON ISSUES RAISED IN EXAMINATION REPORT

It is positive that the Examining Division is now in a position to acknowledge novelty and inventive step for a number of the claims. Therefore, the comments below address only the points in the Examination Report in which actual objections are raised.

Regarding item 3.1, claim 7 has been amended in line with the Examining Division's suggestion.

Regarding item, 5.2, the possibility that both primers recognise SEQ ID NO: 22 has now been removed from the claims. It is also noted that claim 2 relates to "a kit for identifying event TC1507 ... which detects a TC1507 specific region ...", which makes it clear that all kits covered by claim 2 must be effective to perform those functions and excludes any notionally inoperative embodiments.

Regarding items 5.7 and 5.8, claims 27-30 have been cancelled.

Regarding item 5.9, reference to SEQ ID NOS: 19 and 20 has been removed from claim 31 (new claim 27).

On this basis, it is submitted that the Application is now in condition for grant.

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. The Examining Division is also asked to note that the Applicant does not consent to amendments being entered by the Examining Division. If the Examining Division proposes to issue a Communication under Rule 71(3) EPC to allow the Application for grant, but feels that further amendments are needed, the Examiner is requested to telephone me to discuss those beforehand. In particular, as the Examining Division will appreciate, amendments entered to the claims by the EPO at the allowance stage can cause difficulties with the claim translations if the Applicant is not able to agree to the changes.

Yours faithfully

A. BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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.

29. The pair of isolated DNA sequences of claim 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

30. 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.

31. The method of claim 30 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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, 49, 50, 51, 52, 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.

33. The DNA detection kit of claim 11 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. 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, and ~~further comprising at least:~~

   ~~(a)~~ a second primer which recognizes ~~a sequence within~~ SEQ ID NO:25~~, or~~,

   ~~(b) a second primer which recognizes a sequence within SEQ ID NO:22.~~

3. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit ~~specific for junction DNA of maize event TC1507 and its progeny~~ comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is 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~~.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:22.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

~~27. A method of producing an insect resistant corn plant comprising breeding with a plant,~~

~~wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the plant's genome,~~

~~and selecting progeny by analyzing for at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.~~

~~28. A method of claim 27, further comprising obtaining a descent plant of said insect resistant corn plant of claim 27, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.~~

~~29. A method of claim 27 further comprising obtaining seed from said insect resistant corn plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the seed's genome.~~

~~30. A method of claim 28 further comprising obtaining seed from said descent plant, wherein DNA having at least one nucleotide sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof forms part of the descent plant's genome.~~

~~31~~27. 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: ~~19, 20,~~ 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

~~32~~28. 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.

~~33~~29. The pair of isolated DNA sequences of claim ~~32~~ 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

~~34~~30. 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.

~~35~~31. The method of claim ~~34~~ 30 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

~~36~~32. 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, 49, 50, 51, 52, 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.

~~37~~33. The DNA detection kit of claim 11 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

~~38~~34. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

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 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 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 relates to 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.

The invention also relates to 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 are provided.

In addition, a kit and methods for identifying event TC1507 in a biological sample are provided.

In such kits, 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.

The invention also relates to 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 invention also relates to 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 invention therefore provides an isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO: 21, 22, 24, 26 or 27.

The invention also provides a kit for identifying event TC1507 in a biological sample which detects a TC1506 specific region, said kit comprising a first primer which recognises SEQ ID NO: 21 or SEQ ID NO: 22, and a second primer which recognises SEQ ID NO: 25.

The invention also provides a DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

The invention also provides 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.

The invention also provides 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.

The invention also provides a 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

The invention also provides a method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognises a sequence within SEQ ID NO: 21 or SEQ ID NO: 22.

The invention also provides 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

The invention also provides an isolated DNA molecule comprising the amplicon produced by said method.

The invention also provides 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.

The invention also provides an isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

The invention also provides 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, or 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.

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

The invention also provides 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.

The invention also provides 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.

The invention also provides 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

The invention also provides 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.

The invention also provides 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.

The invention also provides 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, 49, 50, 51, 52, 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.

The invention also provides a method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

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.

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 relates to ~~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.

The invention also relates to ~~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 are provided. ~~, 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.

In such kits,
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.

The invention also relates to
~~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 invention also relates to
~~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.~~

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.

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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.

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Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	1109308
Application number	EP04751097.9
Date of receipt	22 February 2011
Receiving Office	European Patent Office, The Hague
Your reference	N.96314 AB
Applicant	All applicants as on file

Documents submitted		
	package-data.xml	ep-sfd-request.xml
	epf1038.pdf (2 p.)	RFPR-1.pdf\N96314 EP AB mr Response with FP - Feb 11.pdf (2 p.)
	CLMSPAMD-1.pdf\N96314 EP AB mr Amended claims - Feb-11.pdf (5 p.)	CLMSPAMD-2.pdf\N96314 EP AB mr [Tracking] Amended claims - Feb-11.pdf (6 p.)
	DESCPAMD-1.pdf\N96314 EP AB mr Amended pages 4, 5, 6 and 7 - Feb 11.pdf (9 p.)	DESCPAMD-2.pdf\N96314 EP AB mr hand amended pgs 4-7.pdf (5 p.)

Submitted by	CN=A. Bentham 23464,O=J A Kemp & Co.,C=GB
Method of submission	Online
Date and time receipt generated	22 February 2011, 16:53 (CET)
Message Digest	33:E6:61:76:A0:E7:2E:EE:3C:FE:E8:C1:EB:B8:15:D9:3A:19:42:FE

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).

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Date: 07-03-2011

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402 / 1620571
Applicant/Proprietor: Dow AgroSciences LLC, et al	

Decision on the request for further processing under Rule 135(3) EPC

The request for further processing received on 22.02.11 has been granted (Art. 121(2) EPC).

☑ The legal consequence notified in the communication dated 02.02.11 that the application was deemed to be withdrawn shall not ensue.

☐ The refusal of the application dated . . . . . shall not ensue.

☐ The legal consequence notified in the communication dated . . . . . that the particular loss of rights occurred shall not
- ☐ ensue.
- ☐ ensue for the following contracting state(s):

☐ The time limit set in the communication dated . . . . . is deemed to have been met.

The procedure shall be continued/the particular loss of rights shall not ensue (Art. 121(3) EPC).

For the Examining Division

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Registered letter
EPO Form 2010 12.07 (02/03/11) RFPR4=3

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Application No.	Ref.	Date
04 751 097.9 - 2402	N.96314 AB	22.11.2011
Applicant: Dow AgroSciences, LLC		

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).

Failure to comply with this invitation in due time will result in the application being deemed to be withdrawn (Art. 94(4) EPC).

Helliot, Bertrand
Primary Examiner
For the Examining Division

Enclosure(s): 4 page/s reasons (Form 2906)
D5: EP1167531 A1 (MONSANTO TECHNOLOGY LLC [US]) 2 January 2002 (2002-01-02).

Registered Letter
EPO Form 2001 12.10CSX

The examination is being carried out on the **following application documents**

Description, Pages

1-3, 8-12, 15-22, 24-43	as published			
13, 14, 23, 44	received on	08-06-2007	with letter of	07.06.2007
4-7, 7a-7e	received on	22-02-2011	with letter of	22-02-2011

Claims, Numbers

1-34	received on	22-02-2011	with letter of	22-02-2011

Drawings, Sheets

1/1	as published

1 INTRODUCTION

1.1 When drafting this communication, the Examining Division has taken the arguments provided by the Applicant in his reply of February, 22nd 2011, into consideration.

1.2 In view of the amended claims, the following document (D5), is referred to in this communication; the numbering will be adhered to in the rest of the procedure:

D5 EP1167531 A1 (MONSANTO TECHNOLOGY LLC [US]) 2 January 2002 (2002-01-02). Corn transformant PV-ZMGT32 (NK603) and compositions and methods for detection thereof.

2 ART. 123(2) EPC

The amendments filed with the letter dated February, 22nd 2011 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 NOVELTY (Art. 52(1) and 54(1) (2) EPC)

As already acknowledged in the previous communication, no cited prior-art document discloses the maize TC1507 event of the present application. Therefore, the subject-matter of **claims 1-34** is considered novel (Art. 54(1) and (2) EPC).

3.1 INVENTIVE STEP (Art. 52(1) and 56 EPC)

In the communication dated January, 9th 2007, the Examining Division opined that the subject-matter of **claim 1** did not involve an inventive step over D1 (Art. 52(1) and 56 EPC).

D1 relates to the same transgenic maize line TC1507 as that one disclosed in the present application. However, no sequence is disclosed therein.

In his reply dated June, 7th 2007, the Applicant disagreed with the above objection arguing that the invention resides in the provision of means for detecting the TC1507 event. The solution to the problem of providing such detection means lay in the difficult and non-obvious process of locating and characterizing the TC1507 within the transformed maize genome.

However, the Examining Division does not agree with the Applicant's contention as the claimed solution to this problem is regarded as obvious because the skilled person, in possession of any particular transgenic plant employs only routine and well established methods to arrive at nucleic acid

sequences comprising said junction regions. The skilled person could and would turn to prior art disclosing detection of known events and apply this teaching analogously to any new event he wishes to detect and particularly to the maize TC1507 event of D1.

Thus, as example of prior art disclosing detection of known events, the skilled person could have turned to D5. D5 provides a DNA construct that confers tolerance to transgenic corn plant. Also provided are assays for detecting the presence of the PV-ZMGT32(nk603) corn event based on the DNA sequence of the recombinant construct inserted into the corn genome and of genomic sequences flanking the insertion site (see [0008]). DNA molecules are provided that comprise at least one junction sequence of PV-ZMGT32(nk603) 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 and is diagnostic for the event ([0008]). DNA event primer pairs and probes are disclosed as well ([0017]).

After reconsideration, the Examining Division is of the opinion that present **claims 1-34** lack an inventive step (Art. 56 EPC).

3.2 For the same reasons as set out under item 5.2 in the previous communication dated June, 15th 2010, the Examining Division is of the opinion that the aforedisclosed problem is not solved when the forward primer and the reverse primer are both selected from the sequence SEQ ID N° 22. Therefore, present **claims 13 and 15** are not inventive (Art. 56 EPC).

4 CLARITY (Art. 84 EPC)

4.1 Present **claim 31** lacks clarity because the primer pairs cannot be selected from the group consisting of sequences as disclosed therein.

4.2 The same applies to **claim 33**.

5 CONCLUDING REMARKS

5.1 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.

5.2 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).

5.3 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.

5.4 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 • KEMP

M. 12.03.12

E-MAIL

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

12 March 2012

Dear Sir, Madam,

For the attention of the CDR department
Our ref: Change of Name to J A Kemp

As from 12 March 2012, this firm has changed its name from "J A Kemp & Co" to "J A Kemp". Our email and website address, telephone numbers, fax numbers and mailing address remain the same.

Please kindly update all applications on the register to reflect the change of name.

Yours faithfully,

ANDREW WEBB
PARTNER
J A KEMP

S 1 FREP

E3,he/cdc

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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

04751097.9 - 2402 / 1620571 14.03.12

Client Database System (CDS) - clean up.

Application Nr.: 04751097.9

Following clean up action in CDS the entries concerning the **Representative for the applicant** have been amended and are now as follows:

Bentham, Andrew
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Where appropriate, the Register of European Patents will be updated to show the amended details.

For questions please contact the Client Data Registration department of the European Patent Office in Munich, telephone +49 (0)89 2399 2780.

EB21171

EPO Form 2596C 04.08

Europäisches Patentamt
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Letter accompanying subsequently filed items

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The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	04751097.9
Applicant's or representative's reference	N.96314 AB

	Description of document	Original file name	Assigned file name
1	Request for extension of time limit during examination procedure	N96314 EP AB mr Extension request - Mar12.pdf	EXRE92-1.pdf

Signatures

Place:

Date: **19 March 2012**

Signed by: **GB, J A Kemp & Co., A. Bentham 23464**

Capacity: **(Representative)**

J A • K E M P

BY ONLINE FILING

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

19 March 2012

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES, LLC
Our ref: N.96314 AB/mr

I refer to your Communication of 22 November 2011 and request a two month extension to the period for response, taking the total response period to six months. Please confirm in due course that this extension has been granted.

Yours faithfully



A BENTHAM
AUTHORISED REPRESENTATIVE

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Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	1549018
Application number	EP04751097.9
Date of receipt	19 March 2012
Receiving Office	European Patent Office, The Hague
Your reference	N.96314 AB
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml EXRE92-1.pdf\N96314 EP AB mr Extension request - Mar12.pdf (1 p.)
Submitted by	CN=A. Bentham 23464,O=J A Kemp & Co.,C=GB
Method of submission	Online
Date and time receipt generated	19 March 2012, 17:45 (CET)
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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).

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For any questions about this communication:
Tel.:+31 (0)70 340 45 00

Date: 23.03.12

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 2402 / 1620571

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 22.11.11 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, part 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).

For the Examining Division

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Europäisches Patentamt
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Letter accompanying subsequently filed items

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The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	04751097.9
Applicant's or representative's reference	N.96314 AB

	Description of document	Original file name	Assigned file name
1	Reply to examination report	N96314 EP AB mr Response to Examination Report - May12.pdf	EXRE3-1.pdf
2	Amended claims (clean copy)	N96314 EP AB mr Amended clean claims - May12.pdf	CLMS-1.pdf
3	Amended claims with annotations	N96314 EP AB mr Amended track change claims - May-12.pdf	CLMS-HWA-1.pdf

Signatures

Place:

Date: **29 May 2012**

Signed by: **GB, J A Kemp & Co., A. Bentham 23464**

Capacity: **(Representative)**

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

29 May 2012

Dear Sirs

European Patent Application No. 04751097.9-2402
DOW AGROSCIENCES, LLC, et al
Our ref: N.96314 AB/mr

This is in response to the Examination Report dated 22 November 2011. On behalf of the Applicant, my comments are set out below.

AMENDMENTS

Please find attached new claims in track-changes and retyped formats.

Claim 13 has been amended to remove the alternative in which the second primer recognises a sequence within SEQ ID NO: 22.

Claim 15 has been amended in a similar way.

Claim 14 now reflects the first option within claim 13 (first primer recognises sequence within SEQ ID NO: 21, second primer within SEQ ID NO: 25) and claim 15 embodies the second alternative (first primer recognises sequence within SEQ ID NO: 22, second primer within SEQ ID NO: 25).

Claims 31 and 33 have been amended to render explicit what was already implicit, i.e. that each member of the primer pair is selected from the group of recited sequences.

COMMENTS ON ISSUES RAISED IN EXAMINATION REPORT

The Applicant's comments on the Examining Division's points are set out below.

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Regarding items 2 and 3 of the Examination Report, it is noted that no issues arise under Articles 123(2) or 54 EPC.

Regarding items 4 and 5.3, the amendments discussed above relate to simple deletions of alternatives and in claims 13/15 and clarification of what the Examining Divisional already understood was the meaning of claims 31/32, so no further comments are believed to be necessary.

Regarding item 5.4, the number and structure of the claims has not changed so again no comments appear to be necessary.

Regarding item 5.2, it is proposed to defer amendment to the description until an allowable set of claims has been agreed upon. However, if the Examining Division would like an amended description to be submitted immediately, please contact me and I will take care of that.

Regarding inventive step and item 3.2, the alternatives in which both primers recognise the sequences within SEQ ID NO: 22 have been removed from claims 13 and 15.

Regarding item 3.1 of the Examination Report (see also item 5.1), it is submitted that the present Application does indeed involve an inventive step for the reasons set out below.

The Examining Division has seemingly mischaracterised the teaching of D1 and drawn an inappropriate parallel between D5 and the present case. In the Applicant's responses dated 19 September 2006 and 7 June 2007, it has been explained that the inventive step here lies in the difficult and non-obvious process of locating and characterising the TC1507 event within the transformed maize genome. The Examining Division takes the view that this is obvious because TC1507 is "known" from D1. It is correct that D1 relates to the same transgenic maize line as the present Application but it is wrong to consider the TC1507 event "known" from D1 in the sense the Examining Division is assuming. Nor is the situation here parallel to that in D5.

In D5, the starting point is a plant in which the **junction sequences surrounding the event are available to the skilled person**. Therefore, it is correct that, with that knowledge, the skilled person can detect the event by routine techniques. For example, the skilled person can employ probes and primers designed based on the knowledge of the junction sequences. (Probes/primers based on the sequence of the cassette in between the junction sequences are of course no use in this regard because those would only identify the generality of plants transformed with the cassette, not the specific event in question, which is defined by the junction sequences where the insert meets wild-type genome.)

In the present case, **the junction sequences are not known from D1**. Therefore, the skilled person cannot employ the techniques used in D5 because those are predicated on a knowledge of the junction sequences that one is looking for. D5 does relate to the same transgenic maize line but it does not disclose enough about that line for the techniques of D5 to be employed because it does not provide the junction sequence information that is decisive to identifying the TC1507 event.

Therefore, all of the Applicant's existing arguments relating to inventive step remain relevant and it is submitted that Article 56 is in fact complied with.

If the Examining Division remains of the view that Article 56 is not complied with, it is requested to set out how, in process terms, it considers that the skilled person could carry out detection of TC1507 based on the disclosure of D1 and in the absence of knowledge of the junction sequences as in D5. Also, it is noted that the Applicant drew attention to the lack of the junction sequence information in D1 as early as the 19 September 2006 response to the EPO's first Examination Report. Therefore, if the Examining Division is having difficulties appreciating the critical relevance of the junction sequences and the lack of knowledge of them from D1 (contrasting with the knowledge of the junction sequences in D5), the Examiner is welcome to give me a call to discuss if that will be of assistance.

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. The Examining Division is also asked to note that the Applicant does not consent to amendments being entered by the Examining Division. If the Examining Division proposes to issue a Communication under Rule 71(3) EPC to allow the Application for grant, but feels that further amendments are needed, the Examiner is requested to telephone me to discuss those beforehand. In particular, as the Examining Division will appreciate, amendments entered to the claims by the EPO at the allowance stage can cause difficulties with the claim translations if the Applicant is not able to agree to the changes.

Yours faithfully



A BENTHAM

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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: 25.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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.

29. The pair of isolated DNA sequences of claim 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

30. 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.

31. The method of claim 30 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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, 49, 50, 51, 52, 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.

33. The DNA detection kit of claim 11 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2(a), wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2(b), wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:~~22~~25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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.

29. The pair of isolated DNA sequences of claim 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

30. 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.

31. The method of claim 30 wherein each member of ~~said~~ the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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, 49, 50, 51, 52, 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.

33. The DNA detection kit of claim 11 wherein each member of ~~said~~ the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

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Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	1669384
Application number	EP04751097.9
Date of receipt	29 May 2012
Receiving Office	European Patent Office, The Hague
Your reference	N.96314 AB
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) CLMS-1.pdf\N96314 EP AB mr Amended clean claims - May12.pdf (6 p.) ep-sfd-request.xml EXRE3-1.pdf\N96314 EP AB mr Response to Examination Report - May12.pdf (3 p.) CLMS-HWA-1.pdf\N96314 EP AB mr Amended track change claims - May-12.pdf (5 p.)
Submitted by	CN=A. Bentham 23464,O=J A Kemp & Co.,C=GB
Method of submission	Online
Date and time receipt generated	29 May 2012, 19:09 (CEST)
Message Digest	3F:89:67:79:59:F0:7C:0A:A0:3C:DC:D9:77:04:B1:69:D7:DF:2D:45

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).

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Application No.: 04 751 097.9

Preparation for oral proceedings - Instructions to Support Service

Oral proceedings are to be held in connection with the above patent application

1. The matters to be discussed are set out in the annex (Form 2906)

2. Dispatch the summons using Form 2008/2310 and Form 2906 for the parties to attend on:

Day 10.09.2014 Time 09:00

ROOMS

Room booked

ORAL 01, 02, 03 and 05 coded

20.02.14 HC
Date Initials

2.1 Parties' submissions in preparation for the oral proceedings, if any, should be made no later than

1 month(s)

before the date of the oral proceedings
(transfer to Form 2008.1 /2310.1)

2.2 Encode ORAL(04) coded

20.02.14 HC
Date Initials

2.3 Dispatch Form 2008.7 /2310.7 to division

20.02.14 HC
Date Initials

3. ☐ Arrange for the following special equipment to be provided in the conference room:

Date Initials

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4. Request language service to provide simultaneous interpretation facilities as necessary

....
Date Initials

5. Return the dossier to primary examiner with Form 2041 (15 days before the oral proceedings)

....
Date Initials

6. Check that summons has been received (Form 2936 / advice of delivery)

7. 15 days before the oral proceedings:
- dispatch the dossier to the primary examiner.

07/02/14
Date

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Chairman	2nd examiner	1st examiner	Legal member

Enclosure(s):

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Date
25-02-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
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 10.09.14 at 09.00 hrs at the EPO,
PschorrHöfe, Bayerstr. 34, D-80335 München

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 1/2009, 68). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC, see also OJ EPO 10/2008, 471).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to the 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 08.08.14.

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.

1st Examiner:	2nd Examiner:	Chairman:
Helliot B	Jacques P	Leber T

For the Examining Division

Annexes:
Confirmation of receipt (Form 2936)
Communication (EPO Form 2906)

Registered letter with advice of delivery
EPO Form 2008 10.13 [ORAL03=9999] (20/02/14)

to EPO postal service: 20.02.14

The examination is being carried out on the following application documents

Description, Pages

1-3, 8-12, 15-22, 24-43	as published			
13, 14, 23, 44	received on	08-06-2007	with letter of	07.06.2007
4-7, 7a-7e	received on	22-02-2011	with letter of	22-02-2011

Claims, Numbers

1-34	received on	29-05-2012	with letter of	29-05-2012

Drawings, Sheets

1/1	as published

1 INTRODUCTION

1.1 After having carefully considered the arguments provided by the Applicant in his reply of May, 29th 2012, 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 E - IX, 4.4) 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 ART. 123(2) EPC

The amendments filed with the letter dated May, 29th 2012 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 NOVELTY (Art. 52(1) and 54(1) (2) EPC)

3.1 In view of the decision of the Enlarged Board of Appeal G1/92, the Examining Division reconsidered its opinion and is thus submits that the subject-matter of **claims 1, 20, 24 and 27** is anticipated by the disclosure of D1 (Art. 54(1) and (2) EPC).

D1 relates to the transgenic maize line TC1507. However, as pointed out by the Applicant in his letter dated May, 29th 2012, if the maize line TC1507 disclosed in the present application is identical to that one in D1, the junction sequences surrounding the event TC1507 are not available to the skilled person at the relevant date of the application.

Nevertheless, 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.

The Enlarged Board of Appeal stated that the same principle applies mutatis mutandis to any other product.

It also stated therein that where it is possible for the skilled person to discover the composition or the internal structure of the product and to reproduce it without undue burden, then both the product and its composition or internal structure become state of the art."

The first question to be answered is whether the maize TC1507 was available to the public at the relevant date of the present application.

In view of D1, the answer is yes. The Examining Division notes that this point is not disputed by the Applicant in his reply dated dated May, 29th 2012.

The second question to be answered is whether it is possible for the skilled person to identify without undue burden the DNA fragment corresponding to the event TC1507 and comprising the 5'- and 3'-flanking regions thereof.

As already argued in the previous communication, the skilled person, in possession of any particular transgenic plant employs only routine and well established methods to arrive at nucleic acid sequences comprising said junction regions. The skilled person could and would turn to prior art disclosing detection of known events and apply this teaching analogously to any new event he wishes to detect and particularly to the maize TC1507 event of D1.

Thus, as example of prior art disclosing detection of known events, the skilled person could have turned to D5. D5 provides a DNA construct that confers tolerance to transgenic corn plant. Also provided are assays for detecting the presence of the PV-ZMGT32(nk603) corn event based on the DNA sequence of the recombinant construct inserted into the corn genome and of genomic sequences flanking the insertion site (see [0008]). DNA molecules are provided that comprise at least one junction sequence of PV-ZMGT32(nk603) 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 and is diagnostic for the event ([0008]). DNA event primer pairs and probes are disclosed as well ([0017]).

Accordingly, the answer to the second question is yes. The Examining Division holds the view that the skilled person could and would turn to D5 disclosing the technical teaching enabling the identification of the sequences surrounding a transgenic event and then would apply this teaching analogously to the maize TC1507 event, for which the introduced genetic

elements are disclosed in D1, thereby identifying the sequences surrounding the event TC1507 listed in the present application as SEQ ID N° 21, 22, 24, 26 and 27.

The third question to be answered is whether it is possible for the skilled person to reproduce without undue burden the DNA fragment corresponding to the event TC1507 and comprising the 5'- and 3'-flanking region thereof.

Since molecular techniques to reproduce a nucleic acid sequence are well known in the art, the skilled person has at hand the technical teaching enabling to reproduce without undue burden the DNA fragment corresponding to the event TC1507 and comprising the 5'- and 3'-flanking region thereof, the answer to the third question is yes.

In conclusion, the sequences surrounding the TC1507 event listed as SEQ ID N° 21, 22, 24, 26 and 27 become state of the art in view of the disclosure of the TC1507 maize line in D1. Accordingly, the Examining Division is of the opinion that the present application does not meet the requirements of Art. 52 (1) EPC, because the subject-matter of **claim 1** is not new over D1 (Art. 54(1) and (2) EPC).

3.2 For the same reasons, the isolated DNA molecules of **claims 20, 24 and 27** are not new over D1 (Art. 54(1) and (2) EPC).

4 INVENTIVE STEP (Art. 52(1) and 56 EPC)

4.1 It is clear from the description and from the examples that primers and probes used in the present application to identify the TC1507 event were designed from sequences surrounding the TC1507 event and tested to specifically detect different sub-sequences thereof. Due to this fact, the claimed sequences are not associated with any particular technical effect. Accordingly, the only underlying technical problem that can be recognised is the provision of oligonucleotides hybridizing to the sequences surrounding the TC1507 event for its identification. Due to the absence of any particular technical

effect, the provision of the present sequences amounts to nothing more than the mere identification of further sequences belonging to a known sequence by routine methodology.

Consequently, primers, primer pairs and probes of **claims 22, 23, 28-29** are considered to lack an inventive step (Art. 56 EPC).

4.2 Moreover, the Examining Division is of the opinion that the use of said sequences in methods for identifying the TC1507 are not complying with the requirements of Art. 56 EPC as such methods are routinely executed by the skilled person at the relevant date of the application (see D5). Accordingly, the subject-matter of **claims 12-18, 19, 21, 25, 26, 30-31, 32-33 and 34** are not inventive (Art. 56 EPC).

4.3 In the light of the objections put forward under items 2 and 3.1 herein above, for the person skilled in the art, grouping the materials used for a series of known or obvious experiments in the form of a kit is not inventive. Thus, **claims 2-11** do not meet the requirements of Article 56 EPC.

5 CLARITY (Art. 84 EPC)

5.1 A lack of clarity arises from the reference to claim 2(a) and claim 2(b) respectively in present claims 3-4 and 5-6 (Art. 84 EPC).

5.2 **Claim 28** relates to 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.

The Guidelines teach that a claim's subject-matter is normally defined in terms of positive features indicating that certain technical elements are present (see G.L. F-IV, 4.20). In the present case, **claim 28** 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 28** is silent as to any structural feature of the claimed pair of isolated DNA sequences that are used to amplify the TC1507 event. In absence of any structural feature relating to

the said pair of isolated DNA sequences, present **claim 28** 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 sequences to be used for diagnosing the TC1507 event.

6 CONCLUDING REMARKS

In view of the different objections as set out under the different items herein above,no further examination is therefore possible.In the case wherein the different objections should not be overcome, the Applicant is kindly informed that the next communication will be a summon to attend oral proceedings according to Art. 116(1) EPC.

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Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

EPA/EPO/OEB Formblatt/Form/Formulaire : 2008, 2906

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**:

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Letter accompanying subsequently filed items

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The document(s) listed below is (are) subsequently filed documents pertaining to the following application:

Application number	04751097.9
Applicant's or representative's reference	N96314-EP

	Description of document	Original file name	Assigned file name
1	Acknowledgement	N96314-EP AXB KKT mqr Signed ack.pdf	ADVOFDELIVRY-1.pdf

Signatures

Place:

Date: **04 March 2014**

Signed by: **Andrew Bentham 23464**

Capacity: **(Representative)**

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Date: 25-02-2014

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EPA/EPO/OEB Formblatt/Form/Formulaire : 2008, 2906

Empfangsbescheinigung über den Zugang des vorstehend bezeichneten Schriftstücks
Acknowledgement of receipt of the document specified above
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Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	2620867
Application number	EP04751097.9
Date of receipt	04 March 2014
Receiving Office	European Patent Office, The Hague
Your reference	N96314-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml ADVOFDELIVRY-1.pdf\N96314-EP AXB KKT mqr Signed ack.pdf (1 p.)
Submitted by	CN=Andrew Bentham 23464
Method of submission	Online
Date and time receipt generated	04 March 2014, 17:52 (CET)
Message Digest	07:57:8F:E5:79:4F:40:9A:C5:58:8A:4A:7A:BB:A5:7C:1D:D4:32:3B

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).

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Application number	04751097.9
Applicant's or representative's reference	N96314-EP

	Description of document	Original file name	Assigned file name
1	Amended claims with annotations	N96314-EP AXB KKT mqr Main Request claims tracked-Aug14.pdf	CLMS-HWA-1.pdf
2	Amended claims (clean copy)	N96314-EP AXB KKT mqr Main Request claims clean - Aug14.pdf	CLMS-1.pdf
3	Amended claims (clean copy)	N96314-EP AXB KKT mqr Auxiliary Request clean claims - Aug14.pdf	CLMS-2.pdf
4	Amended claims with annotations	N96314-EP AXB KKT mqr Auxiliary Request tracked claims-Aug14.pdf	CLMS-HWA-2.pdf
5	Letter dealing with Oral proceedings	N96314-EP SMW KKT mqr Response - Aug14.pdf	ORAL-1.pdf

Signatures

Place:	**London**
Date:	**07 August 2014**
Signed by:	**Simon Wright 22368**
Association:	**J A Kemp**
Capacity:	**(Representative)**

MAIN REQUEST - CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2~~(a)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2~~(a)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2~~(b)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2~~(b)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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: 25.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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.

29. The pair of isolated DNA sequences of claim 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

30. 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.

31. The method of claim 30 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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, 49, 50, 51, 52, 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.

33. The DNA detection kit of claim 11 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

MAIN REQUEST - CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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: 25.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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.

29. The pair of isolated DNA sequences of claim 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

30. 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.

31. The method of claim 30 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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, 49, 50, 51, 52, 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.

33. The DNA detection kit of claim 11 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

AUXILIARY REQUEST - CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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: 25.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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, wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

29. 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.

30. The method of claim 29 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

31. 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, 49, 50, 51, 52, 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.

32. The DNA detection kit of claim 11 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

AUXILIARY REQUEST - CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2~~(a)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2~~(a)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2~~(b)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2~~(b)~~, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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: 25.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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~~,~~.

~~29. The pair of isolated DNA sequences of claim 28~~ wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

29~~30~~. 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.

30~~31~~. The method of claim 29~~30~~ wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

31~~32~~. 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, 49, 50, 51, 52, 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.

3233. The DNA detection kit of claim 11 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

3334. A method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

J A • K E M P

BY ONLINE FILING

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7 August 2014

Dear Sirs

European Patent Application No. 04751097.9-1404
DOW AGROSCIENCES LLC *et al*.
Our Ref: N96314-EP SMW/KKT/mqr

In response to the Summons to attend Oral Proceedings dated 25 February 2014 (which issued almost two years after I last heard from the EPO) please replace the claims at present on file with the claims of the new Main Request attached. I also attach a First Auxiliary Request and marked-up versions of the current claims, showing the amendments made to arrive at both the Main Request (MR) and the First Auxiliary Request (AR1).

The new claims, together with the enclosed submissions, are intended to address all of the outstanding objections. I hope that the Examining Division (ED) will now be in a position to acknowledge that the claims of the Main Request, or failing that the claims of the First Auxiliary Request, comply with the requirements for patentability. I request that this application proceed to allowance, or at the very least continue in writing, without the need for Oral Proceedings.

Main Request (MR)

Amendments and basis thereof

The amendments should be clear from the enclosed track-changes copy of the claims. Claims 3 to 6 have all been amended to depend on claim 2.

Novelty (Item 3 of the Summons)

The present invention relates to event TC1507 in corn, and associated detection methods. The combination of the exogenous nucleic acid sequence, and the specific

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site of integration in the transgenic maize line, confers a surprising and novel technical feature, namely insect resistance. To fully understand the invention, it is therefore necessary to appreciate that the nucleic acid junctions between exogenous and endogenous sequence, in plant material derived from the TC1507 event, are unique to the event in question.

The ED begins by citing Decision G1/92 of the Enlarged Board of Appeal, stating,

> "that the chemical composition of a product [here the nucleic acid] 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 [without undue burden] irrespective of whether or not particular reasons can be identified for analysing the composition" (emphasis added).

In this case, it is important to realise that the necessary materials **were not available to the public**. The invention could not have been reproduced by the skilled person, let alone without undue burden, at the priority date. With respect, the ED is therefore wrong to apply G1/92 to the present case as the facts are very different.

The ED formulates three questions, but appear to arrive at the wrong answer for each question. The ED's overall assessment of novelty in their Communication dated 22 November 2011 was in fact correct, and for the following reasons, we believe that the ED's subsequent change of stance is flawed.

In response to the Examiner's "first question", **at no point** have the Applicants accepted that the skilled person would be able to access a sample of the plant in question. In fact, we submit that the opposite is true. Indeed, on page 2 of our response dated 29 May 2012, we state that.

> "D1 relates to the same transgenic maize line as the present application **but it is wrong to consider the TC1507 event known from D1** in the sense the Examining Division is assuming" (emphasis added).

The (in)ability of the skilled person to readily obtain any form of physical sample of the plant from D1 is the key issue here. In fact, the plant (or any part thereof) of D1 was **not** publicly available at the priority date. So, while D1 discloses, on paper, TC1507, that plant was not publicly available and so D1 is not enabling.

In response to the Examiner's "second question" it is important to note that the information given in D1 is not sufficient to enable the skilled person, at the priority date, to practice the technical teaching of D1. This is consistent with section G-VI,4 of the Guidelines for Examination, which goes on to state that,

> "[i]t should be noted that a chemical compound [here the DNA molecule], the name or formula of which is mentioned in a prior-art document, is not thereby considered as known, unless the information in the document together, where appropriate, with knowledge generally available on the relevant date of the document, **enables** it to be prepared and separated" (emphasis added).

D1 **does not enable** the skilled person to obtain a physical sample of a TC1507-containing plant on which to carry out the analysis the Examining Division has in mind. Rather, D1 is a mere **paper disclosure** of the existence of TC1507 as part of the regulatory approval process. D1 does not provide any sequence information, nor does it make the plant publicly available. In fact, actual physical samples of TC1507 were not available to the public at the priority date (due to the regulatory process). So while D1 refers to the existence of a plant, the plant itself was **not** publicly available and so could not have been used by the skilled person to arrive at the present invention.

While the Examining Division may well be correct that the skilled person could turn to the disclosure of D5 to provide the junction sequences to be identified, this would only be possible if the skilled person had obtained a physical copy of the plant of D1, or suitable genetic material, for analysis. As discussed above, such physical material (i.e. the plant) was not made publicly available at the priority date. It is therefore wrong for the ED to consider that the TC1507 event was publically available from D1. In other words, in order to fully define the junction regions using the teaching of D5, it would have been essential to have a physical specimen of the plant in question, on which to perform an analysis. This was not possible because plants containing the TC1507 event were not available to the public.

In response to the "third question", a skilled person would need physical access to TC1507 germplasm, or other suitable genetic material derived from the plant, to reproduce the event. This is because even if the junction sequences of the event were known (which they were not) the same event could not routinely be obtained again by transformation. Reproducing the event of the invention in the absence of any physical sample would be almost impossible.

In summary, while D1 was disclosed as part of the regulatory approval process, D1 does not provide any sequence information, and at no point was any physical sample (let alone the junction sequence between the exogenous and endogenous DNA) made available. Therefore, D1 is not an enabling disclosure because to reproduce D1, the skilled person needs access to the physical plant. D5 does not provide the key missing element, i.e. the relevant plant, and therefore the combination of D1 and D5 is still missing the key ingredient. Even D1 and D5 is not enabling for TC1507. Accordingly, the present claims are novel under Article 54 EPC.

Inventive Step (Item 4 of the Summons)

As already discussed, the junction sequences, which form an essential part of the invention, were not publicly available and could not be determined from material that was publicly available at the priority date. Therefore, the technical problem in question is not merely the provision of oligonucleotides hybridising to sequences surrounding the TC1507 event, but it is the identification of the specific event in question. The identification of suitable primer sequences would, at best, only be possible if a physical sample of the plant (or its genomic DNA) were publicly available. As discussed previously, this was not the case at the priority date.

The ED then go on to allege that methods, such as those disclosed in D5, could be routinely applied. However, the ED have failed to recognise that D5 relates to a situation where the junction sequence **is known**. In the present case, the primers relate to detection of an event whose junction sequences **were not known** in the art

at the relevant date. While detecting, locating and characterising the TC1507 event within the transformed maize genome might be possible if samples were available, since samples were not available (to the skilled person), such methods can in no way be regarded as routine.

It follows, therefore, that a kit for identifying the specific event of the present invention, was also not available before the priority date. Given the absence of TC1507 plant material, a skilled person would not be able to arrive at the invention claimed, even with the knowledge of D1 and D5. Hence, all of the claims meet the requirements of Article 56 EPC.

Clarity (Item 5 of the Summons)

Turning to clarity, present claims 3 to 6 have been amended to make it clear that they depend on claim 2. Accordingly, the clarity objection to these claims no longer applies.

Regarding the functional definition of the isolated DNA sequences, in this instance, such a definition should be allowable as the sequences needed to practice claim 28 can be deduced from the junction sequence information provided in the specification. Examples of such sequences are disclosed in the application, and the skilled reader would appreciate that other sequences could be used for the same function, in line with section F-IV 6.5 of the Guidelines for Examination. Accordingly, claim 28 is clear within the meaning of Article 84 EPC.

First Auxiliary Request (AR1)

Amendments and basis thereof

The amendments should be clear from the enclosed track-changes copy of the claims, together with the comments provided below.

As with the Main Request, claims 3 to 6 have been amended to depend on claim 2. In addition, previous claims 28 and 29 have been combined to form new claim 28. Basis for this amendment can be found in claims 56 to 58 of International Application PCT/US2004/013538 as filed. Subsequent claims have been re-numbered and re-appended accordingly.

Comments

The above comments regarding the Main Request apply equally *mutatis mutandis* to the first Auxiliary Request. In the claims of AR1, previous claim 28 has additionally been combined with previous claim 29 to form new claim 28. In doing so, the isolated DNA sequences have been explicitly disclosed in terms of their structure; in other words that of SEQ ID NOS: 26 or 27. Accordingly, new claim 28 is clear within the meaning of Article 84 EPC.

Conclusion

For the avoidance of doubt, should the Examining Division still be minded to reject the application, the request for Oral Proceedings is maintained. However, this being the case, and given that there is over a month until the scheduled date of the Oral

J A • K E M P

Proceedings, I would appreciate if the Examining Division would provide detailed oral and/or written arguments as to why they have maintained Oral Proceedings, especially regarding the issues of novelty and inventive step, in view of the present written submissions.

However, I trust that in the light of these submissions, the Examining Division is now in a position to cancel the Summons to Oral Proceedings. This application can then proceed to allowance, or return to a written procedure, on the basis of the Main Request, or failing that the First Auxiliary Request.

Yours faithfully

Electronically Signed
A BENTHAM

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	2930972
Application number	EP04751097.9
Date of receipt	07 August 2014
Receiving Office	European Patent Office, The Hague
Your reference	N96314-EP
Applicant	All applicants as on file

Documents submitted	
package-data.xml	ep-sfd-request.xml
epf1038.pdf (1 p.)	CLMS-HWA-1.pdf\N96314-EP AXB KKT mqr Main Request claims tracked-Aug14.pdf (6 p.)
CLMS-1.pdf\N96314-EP AXB KKT mqr Main Request claims clean - Aug14.pdf (6 p.)	CLMS-2.pdf\N96314-EP AXB KKT mqr Auxiliary Request clean claims - Aug14.pdf (6 p.)
CLMS-HWA-2.pdf\N96314-EP AXB KKT mqr Auxiliary Request tracked claims-Aug14.pdf (6 p.)	ORAL-1.pdf\N96314-EP SMW KKT mqr Response - Aug14.pdf (5 p.)

Submitted by	CN=Simon Wright 22368
Method of submission	Online
Date and time receipt generated	07 August 2014, 16:47 (CEST)
Message Digest	0B:24:62:58:4B:C9:20:72:26:22:4D:19:A9:B7:68:66:F0:F9:D9:9E

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/

European patent application No. 04751097.9

II. To the Formalities Officer

1. ☒ The date / time fixed for oral proceedings is maintained.

   ☐ (if necessary) The reasons are indicated on enclosed EPO Form 2906/2906O, which is to be dispatched to the party with EPO Form 2008A/2310A.

2. ☐ At the instigation of the division the oral proceedings cannot take place on the arranged date for the reasons indicated on enclosed EPO Form 2906, which is to be dispatched to the party with EPO Form 2008A/2310A.

   Date _____ Director _____

3. ☐ The summons to attend oral proceedings on 10.09.14 should be cancelled.

   3.1 ☐ The proceedings will be continued in writing.

   3.2 ☐ A new date will be set later.

   3.3 ☐ A new date/time is set as follows:

   date ..... at ..... hrs.
   Parties' written submissions and amendments in preparation for the oral proceedings, if any, should be made not later than

   ..... month(s) before the date of the oral proceedings.

4. ☐ The request for the oral proceedings to be held as a videoconference

   4.1 ☐ is allowed.

   4.2 ☐ is rejected for the reasons indicated on enclosed EPO Form 2906, which is to be dispatched to the party with EPO Form 2008A.

5. ☐ The application is deemed to be withdrawn. The oral proceedings are cancelled.

11/8/14
Date

Chairman _____ Second member _____ Primary Examiner _____ Legal member _____

III. Action taken by the formalities officer

☒ EPO Form 2008A/~~2310A~~ has been dispatched, if applicable (see point II above) together with EPO Form 2906/2906O.

☐ A new summons has been dispatched.

☐ .....

11.08.14
Date

Formalities Officer

EPO Form 2088 06.14

RM22834

Europäisches Patentamt
European Patent Office
Office européen des brevets

European patent application No. 04751097.9

URGENT !
To be completed (under II.) and returned to the formalities officer immediately

Maintenance / Change of date / Cancellation of oral proceedings arranged for: day 10.09.14 at 09.00 hrs

I. To the Examining / ~~Opposition~~ division

1. ☐ The proprietor has requested the revocation (or the like) of his patent (date: .....).

2. ☒ The party / witness JA KEMP has indicated (date: 07.08.2014) that he / she

   ☐ requests the date / time of oral proceedings to be changed.

   ☐ withdraws the request for oral proceedings.

   ☐ will not be attending.

   ☐ wishes to be heard by a national court.

   ☒ has filed amendments (in examination proceedings).

   ☐ has requested that the oral proceedings be held as a videoconference (in examination proceedings).

3. ☐ Two months before the oral proceedings the application is deemed to be withdrawn (in examination proceedings). The oral proceedings have to be cancelled.

08-08-2014
Date

Moreno, Ramon
Formalities Officer

EPO Form 2088 06.14

RM22834

Europäisches Patentamt
European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
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Fax: +49 89 2399 4465

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME-UNI

Formalities Officer
Name: Moreno, Ramon
Tel: +49 89 2399 - 2658
or call
+31 (0)70 340 45 00

Substantive Examiner
Name: Helliot, Bertrand
Tel: +49 89 2399 - 7793

Application No.	Ref.	Date
04 751 097.9 - 1404	N.96314 AB	14.08.2014
Applicant: Dow AgroSciences, LLC		

Result of consultation

A copy of the result of consultation of 11.08.2014 is enclosed for your information.

Helliot, Bertrand
For the Examining Division

Enclosure(s): Copy of result of consultation (Form 2036)
XP055134128, XP055134135

Europäisches Patentamt
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Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY
Tel: +49 89 2399 0
Fax: +49 89 2399 4465

Application No. : 04 751 097.9

Consultation by telephone with the applicant / representative

Despatch for information

Participants

Applicant: Dow AgroSciences, LLC

Representative: Bentham, Andrew

Member(s) of the Examining Division: Helliot, Bertrand

Result of consultation

See Separate Sheet

11.08.2014
. . . . . .
Date

Helliot, Bertrand
. . . . . .
Examiner

Enclosure(s):

The Applicant (Mr Bentham) was informed that the Examining Division is not convinced by his argument since Mr Bentham fails to provide evidence as to when the TC1507 corn (Herculex 1) was brought to the market and thus publicly available.

D6 (page 1, para. 3) teaches that TC1507 corn was authorized in the United States in 2001 whereas D7 teaches that the same corn line was commercialized in 2003 (page 2).

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Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

Questions about this communication ?
Contact Customer Services at www.epo.org/contact

Formalities officer: Mr Ramon Moreno

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date: 18-08-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
Applicant/Proprietor: Dow AgroSciences, LLC, et al	

BRIEF COMMUNICATION

Oral Proceedings on 10.09.14

Subject: ☐ Your letter of

Communication:
- ☐ The summons to attend oral proceedings on 10.09.14 has been cancelled.
- ☐ The procedure will be continued in writing.
- ☒ The date fixed for oral proceedings is maintained.
- ☐ A new date will be set later.
- ☒ Please confirm receipt of this communication by return fax. Thank you.

Please take note.

For the Examining Division

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Registered letter
EPO Form 2008A 12.07 (11/08/14)

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Fax: +49 89 2399 4465

Bentham, Andrew
J A Kemp
14 South Square
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London WC1R 5JJ
ROYAUME-UNI

Formalities Officer
Name: Smajda, Iveta
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or call
+31 (0)70 340 45 00

Substantive Examiner
Name: Helliot, Bertrand
Tel: +49 89 2399 - 7793

Application No.	Ref.	Date
04 751 097.9 - 1404	N.96314 AB	21.08.2014
Applicant: Dow AgroSciences, LLC		

Result of consultation

A copy of the result of consultation of 20.08.2014 is enclosed for your information.

Helliot, Bertrand
For the Examining Division

Enclosure(s): Copy of result of consultation (Form 2036)

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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. : 04 751 097.9

Consultation by telephone with the applicant / representative

Despatch for information

Participants

Applicant: Dow AgroSciences, LLC

Representative: Bentham, Andrew

Member(s) of the Examining Division: Helliot, Bertrand

Result of consultation

See Separate Sheet

20.08.2014
. . . . . .
Date

Helliot, Bertrand
. . . . . .
Examiner

Enclosure(s):

The Applicant (Mr Bentham) is informed that the Examining Division is convinced by his arguments left on the answering machine of the first Examiner. Therefore, in order to give him the time necessary to find the relevant document concerning the commercialization in the United States of America of the TC1507 corn line or the conditions of said commercialization, Mr Bentham is informed that the summons to attend oral proceedings on 10.09.2014 are cancelled and a new date is set as indicated on the EPO form 2088.

European patent application No. 04751097.9

II. To the Formalities Officer

1. ☐ The date / time fixed for oral proceedings is maintained.

   ☐ (if necessary) The reasons are indicated on enclosed EPO Form 2906/2906O, which is to be dispatched to the party with EPO Form 2008A/2310A.

2. ☐ At the instigation of the division the oral proceedings cannot take place on the arranged date for the reasons indicated on enclosed EPO Form 2906, which is to be dispatched to the party with EPO Form 2008A/2310A.

Date _____ Director _____

3. ☒ The summons to attend oral proceedings on 10.09.14 should be cancelled.

   3.1 ☐ The proceedings will be continued in writing.

   3.2 ☐ A new date will be set later.

   3.3 ☒ A new date/time is set as follows:

   date 28/11/14 at 9.00 hrs.
   Parties' written submissions and amendments in preparation for the oral proceedings, if any, should be made not later than

   ..... month(s) before the date of the oral proceedings.

4. ☐ The request for the oral proceedings to be held as a videoconference

   4.1 ☐ is allowed.

   4.2 ☐ is rejected for the reasons indicated on enclosed EPO Form 2906, which is to be dispatched to the party with EPO Form 2008A.

5. ☐ The application is deemed to be withdrawn. The oral proceedings are cancelled.

20/8/14.
Date

Chairman ~~Second member~~ ~~Primary Examiner~~ Legal member

III. Action taken by the formalities officer

☒ EPO Form 2008A/~~2310A~~ has been dispatched, if applicable (see point II above) together with EPO Form 2906/2906O.

☐ A new summons has been dispatched.

☒ room cancelled

20. 8. 2014
Date

Formalities Officer

EPO Form 2088 06.14

RM22834

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

Formalities officer: Ms Iveta Smajda

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

The former summons dated 25.02.2014 are superseeded herewith.

Date
25-08-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
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 28.11.14 at 09.00 hrs at the EPO,
PschorrHöfe, Bayerstr. 34, D-80335 München

No changes to the date of the oral proceedings can be made, except on serious grounds (see OJ EPO 1/2009, 68). If you do not appear as summoned, the oral proceedings may continue without you (R. 115(2) EPC, see also OJ EPO 10/2008, 471).

Your attention is drawn to Rule 4 EPC, regarding the language of the oral proceedings, and to the 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 28.10.14.

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.

1st Examiner:	2nd Examiner:	Chairman:
Helliot B	Jacques P	Leber T

For the Examining Division

Annexes:
Confirmation of receipt (Form 2936)
Communication (EPO Form 2906)

Europäisches Patentamt • European Patent Office • Office européen des brevets

Registered letter with advice of delivery
EPO Form 2008 10.13 [ORAL03=9999] (20/08/14)

to EPO postal service: 20.08.14

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

Formalities officer: Ms Iveta Smajda

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date: 25-08-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
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

IB50262

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

Mr Andrew BENTHAM
J A Kemp
14 South Square
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London Greater London WC1R 5JJ
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Phone: +44 20 3077 8600
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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	04751097.9
Applicant's or representative's reference	N96314-EP

	Description of document	Original file name	Assigned file name
1	Acknowledgement	N96314-EP AXB KKT mqr Signed ack.pdf	ADVOFDELIVRY-1.pdf

Signatures

Place:

Date: **01 September 2014**

Signed by: **Andrew Bentham 23464**

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

Formalities officer: Ms Iveta Smajda

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

J A KEMP
REC'D 29 AUG 2014
Action by.....

Date
25-08-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
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 :
29/8/14

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

IB50262

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	2975115	
Application number	EP04751097.9	
Date of receipt	01 September 2014	
Receiving Office	European Patent Office, The Hague	
Your reference	N96314-EP	
Applicant	All applicants as on file	
Documents submitted	package-data.xml epf1038.pdf (1 p.)	ep-sfd-request.xml ADVOFDELIVRY-1.pdf\N96314-EP AXB KKT mqr Signed ack.pdf (1 p.)
Submitted by	CN=Andrew Bentham 23464	
Method of submission	Online	
Date and time receipt generated	01 September 2014, 20:45 (CEST)	
Message Digest	41:E9:22:05:CB:D9:25:80:AA:E7:74:8C:F9:D7:56:17:76:92:31:09	

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
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Letter accompanying subsequently filed items

Representative:

Mr Andrew BENTHAM
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14 South Square
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United Kingdom

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NL-2280 HV Rijswijk
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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	04751097.9
Applicant's or representative's reference	N96314-EP

	Description of document	Original file name	Assigned file name
1	Letter dealing with Oral proceedings	N96314-EP SMW KKT mqr Response re Summons - Oct14.pdf	ORAL-1.pdf
2	Document filed during examination procedure	N96314-EP AXB KKT mqr Executed 2002 Technology Agreement.pdf	EXAM-1.pdf
3	Document filed during examination procedure	N96314-EP AXB KKT mqr 2002 Tech Use Agmt - Dow.pdf	EXAM-2.pdf

Signatures

Place:

Date: **16 October 2014**

Signed by: **Andrew Bentham 23464**

Capacity: **(Representative)**

J A • K E M P

BY ONLINE FILING

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

16 October 2014

Dear Sirs

European Patent Application No. 04751097.9
DOW AGROSCIENCES LLC *et al*.
Our Ref: N96314-EP AXB/KKT/mqr

I refer to the replacement Summons to Oral Proceeding dated 25 August 2014, appointing Oral Proceedings on 28 November 2014. Reference is also made to the Examining Division's Communication dated 14 August 2014, reporting my telephone discussion with the Examiner on 11 August and certain information found by the Examining Division regarding the commercialisation of TC1507 maize of this invention. There was then a further Communication dated 21 August, in relation to which I am grateful to the Examining Division for rescheduling the Oral Proceedings originally appointed for 10 September 2014.

When the Examining Division has had the chance to consider these comments, please could the Primary Examiner telephone me to let me know the Division's thoughts as to whether or not Oral Proceedings are still necessary, and to outline any further information that may be required.

As the Examining Division will appreciate, it is unlikely that we will be able to address satisfactorily questions of fact at Oral Proceedings unless these are put to us in writing beforehand. However, in fact I believe that the information that we have been able to gather so far will be sufficient that the Examining Division can cancel the Oral Proceedings and allow the Application for grant.

BACKGROUND

On behalf of the Applicants, I am now in a position to provide further comments on the factual and legal situation surrounding the commercialisation of products of this invention in the United States in 2003.

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

The Examining Division has suggested that TC1507 maize of this invention is the maize known as Herculex 1, that this genetically modified product was first authorised for sale in the United States in 2001 and actually commercialised for the first time in 2003. The Applicants agree with all of this.

By way of background, there are three Applicants on this Application:

(i) Dow Agrosciences LLC,
(ii) E I Du Pont De Nemours and Company, and
(iii) Pioneer Hi-Bred International, Inc.

Pioneer is a subsidiary of Du Pont and the invention arises as a collaboration between Pioneer/Du Pont and Dow. TC1507 (Herculex 1) maize of this invention was sold by both Pioneer/Du Pont and Dow in 2003. The priority date of this Application is 2 May 2003.

FIRST DATES OF SALE UNKNOWN

What is not clear is exactly when in 2003 the first sales of seed of the invention took place. Specifically, it is not clear when the seed of the invention was first transferred to a grower's property for planting. I appreciate that the Examining Division has a *prima facie* reasonable expectation that, because these were the Applicants' own sales, they will know when these took place. However, notwithstanding diligent investigations by the Patent Departments at both Dow and Pioneer, it has in fact not been possible to ascertain the first sale dates within 2003.

This is for reasons to do with the age of the records, over 10 years, which is a considerable time by the standards of the seed industry. I understand that at least some of the relevant records have now been destroyed in accordance with organisational policies covering the length of time for which files are retained and that the others which it has been possible to access are not precise as to the dates on which physical control of the seed passed from Pioneer or Dow to the growers.

NO PUBLIC DISCLOSURE: GROWER AGREEMENTS

However, a fuller understanding of the commercial arrangements between the Applicants and their customers, the growers, demonstrates that in fact even if seed was transferred to growers' land before the 2 May 2003 priority date, this does not in fact render the seed, or the TC1507 event, available to the public within the meaning of the EPC.

The reason for this is that when growers procure "biotech" seed of this nature, they do not buy it in the simple sense that they are free to do with it what they wish. At a legal level, what the grower acquires is a licence to carry out certain acts with the seed <u>but no others</u>. Please find attached copies of the agreements accompanying seed sales by Pioneer and Dow at the time. Specifically, I attach:

- A "Pioneer Hi-Bred technology agreement" dated October 2002. This is a redacted version of an agreement that was actually signed contemporaneously on 26 November 2002 (i.e. in the winter preceding the 2003 growing season) and is therefore clearly representative of the

agreements used by Pioneer at the relevant time. It refers to Herculex 1 maize in the third line of the first paragraph. As will be appreciated, the information that has been blacked out is related to the identity of the particular purchaser and has been obscured for confidentiality/privacy reasons.

- A Dow Agrosciences "Herculex™ 1 insect protection maize grower agreement". This is again a redacted version of an agreement actually signed at the time, on 26 August 2003.

These agreements both show that, whilst seed did at some point during 2003 leave the physical control of Pioneer/Du Pont and Dow, important elements of legal control were always retained.

Referring to the section of the Pioneer agreement headed "You Agree", it is clear that the grower is only permitted to use the seed to raise a commercial crop, in a single season, and for no 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 himself is also prohibited from using the seed for such purposes. Bearing in mind the subject matter of the present claims, which is shaped around the sequences of the event contained in TC1507 maize, clearly "research" access would be required to determine those sequences, which itself is a necessary pre-requisite for availability to the public within the meaning of the EPC. Therefore, the subject matter of the claims cannot be considered to form part of the public domain just because a grower has seed on his land.

Moreover, because the agreement precludes the grower from crop breeding and seed production activities, as well as from saving seed for replanting, and also from passing seed to anybody else for these purposes, merely providing seed to growers as part of a licensed commercial package does not render publicly available the invention in the sense that it can be freely handled or reproduced by third parties: this would require physical access to the germplasm in contravention of the technology agreement. Nor are the seed/plants of the invention ever present "in public"; they are either within the physical control of the Applicants or held on the grower's private land.

This Dow agreement contains the same provisions, again in the paragraph headed "You Agree".

As the sales took place in the United States, naturally it is US law that governs them (see the governing law clause in capitals under "General Conditions" in the Pioneer agreement and clause 4.08 of the Dow agreement). Similar agreements have on several occasions been successfully enforced in the US courts by seed companies whose customers have failed to respect their terms. More detail regarding US law in this respect can be provided if necessary.

FINAL POINTS

Having regard to my letter of 7 August 2014, it is believed that this resolves the issues of novelty and inventive step raised by the Examining Division. One of the

issues of clarity raised in the original 25 February 2014 Summons has been addressed by amendment (see page 4 of my 7 August letter) and it is submitted that the other one is not in fact problematic (again see page 4 of my 7 August letter), failing which it is addressed by the amendment contained in Auxiliary Request 1 filed on 7 August (again see the 7 August letter for discussion of this).

For these reasons, I hope that it will now be possible to cancel the Oral Proceedings scheduled for 28 November and either issue Communication under Rule 71(3) EPC or continue examination in writing. Regarding the description, some conforming amendments have been entered already and it is believed that no more are required. However, if the only outstanding issue is conformity between the claims and the description, the Examiner is asked to contact me by telephone such that we can either agree amendments that way or I can file amended pages without the need for a further Examination Report.

I look forward to hearing from the Examining Division in due course.

Yours faithfully

Electronically Signed
A BENTHAM

October 2002

0821 [redacted]

SMO Code HP1A

Pioneer Hi-Bred Technology Agreement

DEC – 6 2002

Pioneer Hi-Bred International, Inc.'s Technology Agreement provides you the opportunity to purchase and plant Pioneer® brand products containing or developed with new technologies. This Technology Agreement presently covers Pioneer brand products containing the Roundup Ready[1] gene in corn and soybeans Yieldgard[1] Corn Borer protection in corn, and Herculex[2] I *Insect Protection* technology in corn.

This Agreement, once signed, will remain in effect until terminated in writing by you or Pioneer. As new information and technologies become available, or if new terms are applicable, we will inform you in writing, at least yearly. Continuing to use the technologies, which are subject to this agreement, is an affirmation and an agreement that you are bound by these and any new terms. All new technology requirements will become incorporated by reference into this master agreement. If you have previously signed a technology agreement with Pioneer, this agreement replaces and terminates any previous agreements.

THIS AGREEMENT PROVIDES YOU:

- A Limited License to purchase and plant Pioneer brand corn and soybean products containing the subject technologies described above. See Bag and Tag for important information, which is incorporated by this reference, regarding License rights and restrictions as well as Limitation of Warranty and Arbitration under state seed laws.

YOU UNDERSTAND:

- The technology provided with the product may include patented subjected matter owned by Pioneer or licensed from a third party. A list of applicable patents is set forth below. These technologies are protected under U.S. patent law.
- These technologies can only be used in locations where the products have been approved for use by all required governmental agencies. See the appropriate Product Use Guide(s) for the applicable technologies for more information on their use.
- Certain grain marketing responsibilities are required for some of these products due to their regulatory status outside of the United States.

YOU AGREE:

- To use the seed containing the subject technology for planting a commercial crop only in a single season.
- To not supply any of this seed to any other person or entity for planting, and not to save any crop produced from this seed for replanting, or supply saved seed to anyone for replanting.
- To not use this seed or its progeny or provide it to anyone for crop breeding, seed production or research (other than to make agronomic comparisons and conduct yield testing).
- To implement an Insect Resistance Management (IRM) program as specified in the appropriate Product Use Guide(s) accompanying your products, if applicable, and to cooperate with Insect Resistance Management programs and research. The IRM program requires planting a non-Bt corn refuge, following EPA-mandated use restrictions as outlined in the appropriate Product Use Guide(s). Please refer to the appropriate Product Use Guide(s), which is supplemental to this Agreement, for specifics related to these terms, including refuge size, distances and management requirements.
- That you are affirming your contractual obligation to follow IRM requirements. Failure to follow IRM requirements can result in the grower losing access to Bt corn for at least one year.
- The IRM requirements set forth in Product Use Guides and referred to in this Agreement supersede the IRM requirements set forth in any previously executed agreement or Product Use Guide.

GENERAL CONDITIONS:

- The Grower's rights may not be transferred to anyone else other than a duly authorized agent of Grower without written consent of Pioneer. If the Grower's rights are transferred with Pioneer's consent or by operation of law, this Agreement is binding on the person or entity receiving the transferred rights.
- If the Grower intentionally breaches this Agreement, in addition to other penalties, the Grower's rights under his Agreement will terminate immediately and the Grower forfeits any right to obtain a license to the subject technology in the future. If the Agreement is terminated, you will no longer have a right under this Agreement to purchase seed containing these technologies. Any obligations that arose before termination will continue in effect.

THIS AGREEMENT IS GOVERNED BY THE LAWS OF THE STATE OF IOWA AND OF THE UNITED STATES. THE PARTIES AGREE TO CONSENT TO THE JURISDICTION OF THE U.S. DISTRICT COURT FOR THE SOUTHERN DISTRICT OF IOWA FOR ALL DISPUTES ARISING UNDER THIS AGREEMENT.

If you have any questions about the subject technology or this agreement, contact your seed provider or call Pioneer at 1 (877) 746-6337. For details of the required Insect Resistance Management program and requirements, see the appropriate Product Use Guide(s). A copy is available from your Pioneer seed provider.

* Licensed or applicable U.S. patents include: **for Pioneer brand corn hybrids and soybeans containing the Roundup Ready[1] gene** — 4,940,835; 5,188,642; 5,352,605; 5,530,196; 5,633,435; 5,717,084; 5,728,925; and 5,804,425; **for Pioneer brand corn hybrids containing Yieldgard[1] Corn Borer protection** — 5,500,365; 5,352,605; 5,359,142; 5,322,938; 5,164,316; 5,196,525; 5,424,412 and 5,424,200; **for Pioneer brand corn hybrids containing Herculex[2] I *Insect Protection* technology** — 5,691,308; 5,188,960; 5,538,880; 5,639,877; 5,489,520; 5,550,318; 5,484,956 and 5,919,675.

I, the undersigned Grower, acknowledge that I have read and understand the terms and conditions of this Pioneer Hi-Bred Technology Agreement and that I agree to them. 26/11/02 (Day/Month/Year)

Signature of Grower or Grower's Agent [redacted]

Grower's Printed Name [redacted] Farmer's Identification Number [redacted]

Address [redacted]

City, State, Zip Code Rantoul, IL 61866

County Champaign

Sales Rep Name [redacted] Sales Rep Signature [redacted]

Sales Rep Identification Number [redacted] DEC 1 2 2002

PIONEER. A DUPONT COMPANY

PIONEER® brand products are provided subject to the terms and conditions of purchase, which are part of the labeling and purchase documents. ®, SM, TM Trademarks and service marks, registered or applied for, of Pioneer Hi-Bred International, Inc., Des Moines, Iowa, U.S.A. ©2002 PHI

1 Registered trademark used under license from Monsanto Company.

2 Herculex I Insect Protection technology by Dow AgroSciences and Pioneer Hi-Bred. Herculex is a trademark of Dow AgroSciences LLC. REP AGR

White copy - Return to Pioneer
Yellow copy - Customer Copy

HERCULEX™ I INSECT PROTECTION
CORN GROWER AGREEMENT

Dow AgroSciences

HERCULEX™ I Insect Protection

By signing and returning this Dow AgroSciences Grower Agreement, ("Agreement") you (herein referred to as "you" or "Grower") gain the right to obtain, plant and grow Dow AgroSciences Herculex™ I Insect Protection corn.[1]

In consideration of Dow AgroSciences LLC (herein referred to as "Dow AgroSciences" or "DAS") licensing you to grow DAS Herculex I Insect Protection corn, you agree to the following terms and conditions:

1. **Patent License.** You acknowledge that Dow AgroSciences Herculex I Insect Protection gene technology is protected under U.S. Patent Nos. 5,691,308; 5,188,960; 5,538,880; 5,538,877; 5,489,520; 5,550,318; 5,484,956 and 5,919,675. Upon Dow AgroSciences' receipt of this Agreement unaltered and executed by you, Mycogen Corporation licenses you the right to use the Herculex I Insect Protection technology solely to produce a single commercial corn crop upon the terms and conditions stated herein.

This Agreement only covers the United States and does not authorize planting of seed in the United States which has been purchased in another country or planting of seed in another country which has been purchased in the United States.

You agree:

- not to supply, license or sublicense any seed containing Herculex I to any other person or entity for planting or any other purpose;
- not to save any grain produced from seed containing Herculex I for replanting by you or any other person or entity; or
- not to use seed, grain or any plant material containing Herculex I genetics, or provide such seed, grain or plant material to any other person or entity, for crop breeding, research, generation of registration data, seed production or any other purpose not expressly authorized under this Agreement.

2. **Grower Guide.** A key component of this Agreement is complying with the provisions of the Herculex I Product Use Guide ("Product Guide"). You understand that you must obtain, read and comply with the provisions of the most current Product Guide. The Product Guide provides critical information and requirements relating to:

- Implementing the required Insect Resistance Management program, including refuge requirements and distance requirements;
- Channeling grain to appropriate channels of trade and uses; and
- Restrictions on where Herculex I corn may be planted.

For so long as you have a valid Agreement for Herculex I corn in effect with Dow AgroSciences, DAS will send you the current Product Use Guide as well as periodic updates relating to DAS Herculex I corn. Your use of any DAS Herculex I corn after receipt of any new terms or conditions relating to DAS Herculex I corn constitutes your agreement to be bound by such terms and conditions.

3. **Term and Termination.** This Agreement shall remain in effect until terminated by either you or Dow AgroSciences. Either party may terminate this Agreement at any time for any reason by sending written notice of termination to the other party at the address for such party specified below. In the case of termination by you the Grower, such notice of termination must include your full name and address. If you violate the terms of this Agreement, in addition to all other remedies available to DAS, you may forfeit any right to obtain an Agreement and other technologies from DAS in the future. A violation of this Agreement may also constitute infringement of one or more of the patents that relate to Herculex I. Upon the termination of this Agreement, you may no longer purchase or use seed containing DAS Herculex I technologies, however, your obligations and DAS' rights that arose under the Agreement prior to the termination will continue in effect.

4. **General Provisions.**

4.01 You agree to communicate all applicable terms, conditions and restrictions on your Herculex I corn whether under this Agreement, a Product Guide or otherwise to all persons and entities possessing or taking an interest in your Herculex I corn crop and/or the grain therefrom.

4.02 You consent to Dow AgroSciences and its representatives (i) entering upon any land where you have planted or are growing any Herculex I corn as well as the refuge area therefor for purposes of examining the land, your crop and taking samples thereof; (ii) reviewing FSA crop reporting information including Forms 578 and corresponding aerial photographs; and (iii) obtaining copies of invoices of your seed and chemical transactions from your seed and chemical dealer.

4.03 Your rights under this Agreement may not be transferred to anyone else without the written consent of Dow AgroSciences. If your rights are transferred with DAS' consent or by operation of law, this Agreement shall be binding on the person or entity receiving such transferred rights.

4.04 You agree that DAS shall be entitled to recover any costs or expenses, including reasonable attorneys fees, it incurs in enforcing its rights under this Agreement.

[1]NOTE: This Agreement covers Herculex I Insect Protection corn for seed companies licensed by Dow AgroSciences LLC. This Agreement does not apply to Pioneer brand Herculex I Insect Protection corn. You must obtain a license to plant Pioneer brand Herculex I directly from Pioneer Hi-Bred.

4.05 **Warranties and Disclaimers.** Dow AgroSciences warrants that the Herculex I gene technology licensed hereunder conforms to the written description therefor on the Dow AgroSciences approved Seed Tag affixed to the Herculex I corn seed bag. This warranty applies only to Dow AgroSciences Herculex I gene technology contained in planting seed that has been purchased from a seed company licensed by Dow AgroSciences, or such seed company's authorized dealers or distributors, and planted from the original sealed bag. Dow AgroSciences makes no other warranties of any kind and disclaims all other warranties, oral or written, express or implied including any implied warranties of fitness for particular purpose and merchantability.

4.06 **Notice Requirement.** As a condition precedent to you, or any other person with an interest in your crop, asserting a claim or commencing an action against Dow AgroSciences and/or any seller of seed containing Dow AgroSciences' Herculex I technologies regarding the performance of the gene technologies or the seed in which it is contained, you must provide prompt and timely notice to Dow AgroSciences (regarding performance or non-performance of the gene technologies) and/or the seller of any seed (regarding performance or non-performance of the seed) within sufficient time to allow an in-field inspection of the crop(s) about which any controversy, claim, action, or dispute is being asserted. The notice shall include the Grower's name, address, FSA number, a description of the claim, and the hybrid and lot number of the Herculex I corn seed at issue.

4.07 **Limitation of Remedies and Liability**. Your exclusive remedy and the limit of the liability of Dow AgroSciences or any seller to any party for any and all losses, injury or damages arising from or relating to the use or handling of a product containing Dow AgroSciences Herculex I gene technology (including claims based in contract, negligence, product liability, strict liability or other theories of liability) shall be the price paid by the Grower for the quantity of Herculex I corn seed involved or, at the election of Dow AgroSciences or any seller, the replacement of such quantity of seed. In no event shall Dow AgroSciences or any seller be liable for any incidental, consequential, special, exemplary or punitive damages.

4.08 This Agreement is governed by the laws of the state of Indiana without regard to its choice of laws provisions.

4.09 If any provision of this Agreement is determined to be void or unenforceable, the remaining provisions shall remain in full force and effect.

4.10 The failure of DAS to exercise one or more of its rights under this Agreement on one or more occasions shall not be deemed a waiver on the part of DAS to exercise such right or rights on one or more subsequent occasions.

4.11 If the information you provide to Dow AgroSciences herein or otherwise in connection with Herculex I corn changes, you agree to promptly provide DAS with your updated information at the DAS address provided below.

4.12 **Please mail the signed Grower Agreement to the following address:** **Mycogen Seeds**
P.O. Box 209, 2220 West Lincoln
Olivia, MN 56277
Attn: Herculex I administrator

Please direct any questions about this Agreement or DAS Herculex I Insect Protection to your seed provider or to Dow AgroSciences at 1-877-4-TRAITS (1-877-487-2487). For additional information on Herculex I corn, visit the DAS web site at www.herculex.net.

YOU, THE UNDERSIGNED GROWER, HEREBY ACKNOWLEDGE AND AGREE THAT: (I) YOU HAVE READ AND YOU UNDERSTAND THE TERMS AND CONDITIONS OF THIS AGREEMENT, (II) THIS IS A LEGALLY BINDING CONTRACT, AND (III) YOU ARE VOLUNTARILY ENTERING INTO THIS AGREEMENT WITH THE INTENT TO BE BOUND BY ITS TERMS AND CONDITIONS.

Please print legibly, sign and date. 44 344

[redacted]
Grower's Business Name

August 26 2007
Date

By: [redacted]
Signature of Grower or Grower's Agent

Grower's Phone#

Printed Name and Title of Person Signing

Grower's FAX #

[redacted]
Address

Grower's E-Mail Address

[redacted]
City, State, Zip

Grower Rapid #

County

[redacted]
Dealer Name

Herculex I Insect Protection technology by Dow AgroSciences and Pioneer Hi-Bred
™Herculex and the Herculex Shield Logo are trademarks of Dow AgroSciences LLC
©2002 Dow AgroSciences LLC

Planting Ratepot, Preserving Technology

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	3065318
Application number	EP04751097.9
Date of receipt	16 October 2014
Receiving Office	European Patent Office, The Hague
Your reference	N96314-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) EXAM-1.pdf\N96314-EP AXB KKT mqr Executed 2002 Technology Agreement.pdf (1 p.) ep-sfd-request.xml ORAL-1.pdf\N96314-EP SMW KKT mqr Response re Summons - Oct14.pdf (4 p.) EXAM-2.pdf\N96314-EP AXB KKT mqr 2002 Tech Use Agmt - Dow.pdf (2 p.)
Submitted by	CN=Andrew Bentham 23464
Method of submission	Online
Date and time receipt generated	16 October 2014, 17:43 (CEST)
Message Digest	09:A6:85:82:BE:DA:F3:4F:0C:70:BE:E9:1A:B7:BB:E9:3B:9B:2B:D4

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 application No. 04751097.9

URGENT !
To be completed (under II.) and returned to the formalities officer immediately

Maintenance / Change of date / Cancellation of oral proceedings arranged for:
day 10.09.14 at 09.00 hrs

I. To the Examining / ~~Opposition~~ division

1. ☐ The proprietor has requested the revocation (or the like) of his patent (date: .....).

2. ☒ The party / witness J A KEMP ..... has indicated (date: 16.10.2014 .....) that he / she

   ☐ requests the date / time of oral proceedings to be changed.

   ☐ withdraws the request for oral proceedings.

   ☐ will not be attending.

   ☐ wishes to be heard by a national court.

   ☒ has filed comments & documents ~~amendments~~ (in examination proceedings).

   ☐ has requested that the oral proceedings be held as a videoconference (in examination proceedings).

3. ☐ Two months before the oral proceedings the application is deemed to be withdrawn (in examination proceedings). The oral proceedings have to be cancelled.

17-10-2014
Date

Moreno, Ramon
Formalities Officer

EPO Form 2088 06.14

RM22834

European patent application No. 04751097.9

II. To the Formalities Officer

1. ☐ The date / time fixed for oral proceedings is maintained.

   ☐ (if necessary) The reasons are indicated on enclosed EPO Form 2906/2906O, which is to be dispatched to the party with EPO Form 2008A/2310A.

2. ☐ At the instigation of the division the oral proceedings cannot take place on the arranged date for the reasons indicated on enclosed EPO Form 2906, which is to be dispatched to the party with EPO Form 2008A/2310A.

Date _____ Director _____

3. ☒ The summons to attend oral proceedings on 10.09.14 should be cancelled.

   3.1 ☒ The proceedings will be continued in writing.

   3.2 ☐ A new date will be set later.

   3.3 ☐ A new date/time is set as follows:

   date ..... at ..... hrs.
   Parties' written submissions and amendments in preparation for the oral proceedings, if any, should be made not later than

   ..... month(s) before the date of the oral proceedings.

4. ☐ The request for the oral proceedings to be held as a videoconference

   4.1 ☐ is allowed.

   4.2 ☐ is rejected for the reasons indicated on enclosed EPO Form 2906, which is to be dispatched to the party with EPO Form 2008A.

5. ☐ The application is deemed to be withdrawn. The oral proceedings are cancelled.

Date 23/10/14

Chairman _____ Second member _____ Primary Examiner _____ Legal member _____

III. Action taken by the formalities officer

☒ EPO Form 2008A/2310A has been dispatched, if applicable (see point II above) together with EPO Form 2906/2906O.

☐ A new summons has been dispatched.

☒ room cancelled

23.10.14
Date

Formalities Officer

EPO Form 2088 06.14

RM22834

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

Formalities officer: Ms Iveta Smajda

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date
28-10-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

BRIEF COMMUNICATION

Oral Proceedings on 28.11.14

Subject: ☒ Your letter of 16.10.2014

Communication:
- ☒ The summons to attend oral proceedings on 28.11.14 has been cancelled.
- ☒ The procedure will be continued in writing.
- ☐ The date fixed for oral proceedings is maintained.
- ☐ A new date will be set later.
- ☒ Please confirm receipt of this form. Thank you.

Please take note.

For the Examining Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

Registered letter
EPO Form 2008A 12.07 (23/10/14)

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

Formalities officer: Ms Iveta Smajda

Please specify the name of the formalities officer when contacting Customer Services by contact form or phone.

Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME UNI

Date: 28-10-2014

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571

Applicant/Proprietor: Dow AgroSciences, LLC, et al

BRIEF COMMUNICATION

Oral Proceedings on 28.11.14

Subject: ☒ Your letter of 16.10.2014

Communication:
- ☒ The summons to attend oral proceedings on 28.11.14 has been cancelled.
- ☒ The procedure will be continued in writing.
- ☐ The date fixed for oral proceedings is maintained.
- ☐ A new date will be set later.
- ☒ Please confirm receipt of this form. Thank you.

Please take note.

For the Examining Division

Europäisches Patentamt · European Patent Office · Office européen des brevets

J A KEMP
acknowledge receipt
of your fax
03 OCT 2014
which is receiving our attention

Registered letter
EPO Form 2008A 12.07 (28/10/14)

Duration: 03.11.2014 09:23:37 - 03.11.2014 09:23:48. This page 1 of 1 was completed at 03.11.2014 09:23:48
Received at the EPO on Nov 03, 2014 09:23:48. Page 1 of 1

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80298 MUNICH
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Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
London WC1R 5JJ
ROYAUME-UNI

Formalities Officer
Name: Smajda, Iveta
Tel: +49 89 2399 - 7942
or call
+31 (0)70 340 45 00

Application No.	Ref.	Date
04 751 097.9 - 1404	N.96314 AB	07.01.2015
Applicant: Dow AgroSciences, LLC, et al		

Communication under Rule 71(3) EPC

1. Intention to grant

You are informed that the Examining Division intends to grant a European patent on the basis of the above application with the text and drawings and the related bibliographic data as indicated below.

A copy of the relevant documents is enclosed.

1.1 In the text for the Contracting 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

Description, Pages

1-3, 8-12, 15-22, 24-43	as published			
13, 14, 23	received on	08-06-2007	with letter of	07.06.2007
4-7, 7a-7e	filed in electronic form on			22-02-2011

Sequence listings, SEQ ID NO

1-57	as published

Claims, Numbers

1-34	filed in electronic form on	07-08-2014

Drawings, Sheets

1/1	as published

Registered letter
EPO Form 2004C 06.13TRI

With the following amendments to the above-mentioned documents proposed by the division

Description, Pages 3*, 4*, 6*, 7*, 23**, 24***, 43**

Comments

* G.L. F-IV, 4.3
** G.L. F-IV, 4.4
*** G.L. F-III, 8

1.2 Bibliographic data

The title of the invention in the three official languages of the European Patent Office, the international patent classification, the designated Contracting States, the registered name(s) of the applicant(s) and the other bibliographic data are shown on the **enclosed EPO Form 2056.**

2. Invitation

You are invited, **within a non-extendable period of four months** of notification of this communication,

2.1 to EITHER approve the text communicated above and verify the bibliographic data (R. 71(5) EPC)

(1) by filing a translation of the claim(s) in the other two official languages of the EPO

		Fee code	EUR
(2a)	by paying the fee for grant including the fee for printing (Art. 2(2) RFees):	**007**	915.00
	minus any amount already paid (R. 71a(5) EPC):		0.00
(2b)	by paying the printing fee (Art. 2(2) RFees) for the 36th and each subsequent page; number of pages payable: 41	**008**	615.00
	minus any amount already paid (R. 71a(5) EPC):		0.00
		Total amount:	1530.00
(3)	by paying additional claims fees under Rule 71(4) EPC; number of claims fees payable: 0	**016**	0.00
	minus any amount already paid (R. 71a(5) EPC):		0.00
		Total amount:	0.00

Important: If the translations of the claims and fees have already been filed and paid respectively in reply to a previous communication under Rule 71(3) EPC, e.g. in the case of resumption of examination after approval (see Guidelines C-V, 6), **agreement as to the text to be granted** (R. 71a(1) EPC) must be expressed within the same time limit (e.g. by approving the text and verifying the bibliographic data, by confirming that grant proceedings can go ahead with the documents on file and/or by stating which translations of the claims already on file are to be used).

Note 1: See "Important notes concerning fee payments" below.
Note 2: Any overpaid "minus" amounts will be refunded when the decision to grant (EPO Form

2006A) has been issued.

2.2 OR, in the case of disapproval, to request reasoned amendments or corrections to the text communicated above or keep to the latest text submitted by you (R. 71(6) EPC).

In this case the translations of the claims and fee payments mentioned under point 2.1 above are NOT due.

The terms "amendment(s)" and "correction(s)" refer only to amendments or corrections of the application documents and not of other documents (e.g. bibliographic data, the designation of the inventor, etc.).

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).

2.3 Bibliographic data

Where you request a change or correction of bibliographic data in response to the Rule 71(3) communication, this will **not** cause the sending of a second communication under Rule 71(3) EPC. You will still have to pay the fees and file translations in reply to the Rule 71(3) communication in the case of 2.1 above, unless you also file a reasoned request for amendments or corrections in response to the Rule 71(3) communication (see case 2.2 above).

3. Loss of rights

If neither of the two possible actions above (see points 2.1 or 2.2) is performed in due time, the European patent application will be deemed to be withdrawn (R. 71(7) EPC).

4. Further procedure

4.1 In the case of point 2.1 above

4.1.1 The decision to grant the European patent will be issued, and the **mention of the grant** of the patent will be published in the European Patent Bulletin, if the requirements concerning the translation of the claims and the payment of all fees are fulfilled and there is agreement as to the text to be granted (R. 71a(1) EPC).

Note on payment of the renewal fee:
If a renewal fee becomes due before the next possible date for publication of the mention of the grant of the European patent, publication will be effected only after the renewal fee and any additional fee have been paid (R. 71a(4) EPC).

Under Article 86(2) EPC, the obligation to pay renewal fees to the European Patent Office terminates with the payment of the renewal fee due in respect of the year in which the mention of the grant of the European patent is published.

Note on payment of the designation fee(s):
If the designation fee(s) become(s) due after the communication under Rule 71(3) EPC, the mention of the grant of the European patent will not be published until the designation fee(s) has/have been paid (R. 71a(3) EPC).

4.1.2 After publication, the **European patent specification** can be downloaded free of charge from the EPO publication server https://data.epo.org/publication-server or ordered from the Vienna sub-office upon payment of a fee (OJ EPO 2005, 126).

Until 31 December 2013, each proprietor may request in writing to receive the certificate for the European patent together with one copy of the new patent specification only if the request is filed within three-month or the additional two-month period (R. 95(3) and (4) EPC). If such request has been previously filed, it has to be

confirmed within the same period. The requested copy is free of charge. Any request or confirmation of a request filed after 31 December 2013 or after expiry of these periods will not be dealt with and the certificate will be delivered without a copy of the new European patent specification (cf. Notice from the European Patent Office dated 16 July 2013 concerning communication of the certificate for a European patent, OJ 2013, 418).

4.1.3 Filing of translations in the Contracting States

As regards translation requirements prescribed by the Contracting States under Article 65(1) EPC, please consult the website of the European Patent Office
www.epo.org ➞Law & practice ➞Legal texts, National law relating to the EPC
www.epo.org ➞Law & practice ➞All Legal texts ➞London Agreement

In the case of a valid extension or validation

As regards translation requirements prescribed by the Extension or or Validation States, please consult the website of the European Patent Office
www.epo.org ➞Law & practice ➞Legal texts, National law relating to the EPC

Failure to supply a prescribed translation in a Contracting State, or in an Extension or Validation State may result in the patent being deemed to be void *ab initio* in the State concerned (Art. 65(3) EPC).

4.2 In case of 2.2 above

If the present communication under Rule 71(3) EPC is based on an auxiliary request and, within the time limit, you maintain the main request or a higher ranking request which is not allowable, the application will be refused (Art. 97(2) EPC).

If the Examining Division gives its consent to the requested amendments or corrections, it will issue a new communication under Rule 71(3) EPC; otherwise, it shall resume the examination proceedings (R. 71(6) EPC).

5. Filing of a divisional application

Any divisional application relating to this European patent application must be filed directly with the European Patent Office in Munich, The Hague or Berlin and will be in the language of the proceedings for the present application, or if the latter was not in an official language of the EPO, the divisional application may be filed in the language of the present application as filed (see Article 76(1) and Rule 36(2) EPC). Any such divisional application must be filed while the present application is still pending and the time limit for filing divisional applications must be observed (R. 36(1) EPC; Guidelines A-IV, 1.1.1).

6. Important notes concerning fee payments

6.1 For all payments, please refer to the relevant **fee code(s).**

6.2 Automatic debiting procedure

The fee for grant, including the fee for printing (Art. 2(2) RFees), and any additional claims fees due under Rule 71(4) EPC will be debited automatically on the date of filing of the translations of the claims, or on the last day of the period of this communication. However, if the designation fee(s) become(s) due as set out in Rule 71a(3) EPC and/or a renewal fee becomes due as set out in Rule 71a(4) EPC, these should be paid separately by another permitted way of payment in order not to delay the publication of the mention of the grant. The same applies in these circumstances to the payment of extension fees. For further details see the Arrangements for the automatic debiting procedure (AAD) and accompanying information from the EPO concerning the automatic debiting procedure (Annexes A.1 and A.2 to the Arrangements for deposit accounts (ADA) in Supplementary publication 4 - OJ EPO 2014).

6.3 Important information relating to 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.

6.4 **Payments by cheque** delivered or sent direct to the EPO are no longer accepted as from 1 April 2008 (see OJ EPO 2007, 626).

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Enclosure(s): Text intended for grant (76 pages)

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Annex to EPO Form 2004, Communication pursuant to Rule 71(3) EPC

Bibliographical data of European patent application No. 04 751 097.9

For the intended grant of the European patent, the bibliographical data are set out below, for information:

Title of invention:
- "EVENT 1507" MAIS UND VERFAHREN FUR SEINE DETEKTION
- CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF
- MAIS TC1507 ET PROCEDES DE DETECTION DE CELUI-CI

Classification: INV. C12Q1/68

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Extension States* for which fees have been paid:

Validation States* for which fees have been paid:

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*) If the time limit for the payment of designation fees according to Rule 39(1) EPC has not yet expired and the applicant has not withdrawn any designation, **all Contracting States/Extension States/Validation States** are currently still deemed to be designated.
See also Rule 71a(3) EPC and, if applicable, the above Note to users of the automatic debiting procedure.

**) If two or more applicants have designated different Contracting States, this is indicated here.

~~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~~ 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. ~~According to one aspect of the invention,~~ 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.

~~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);

Druckexemplar

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 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 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 relates to 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.

The invention also relates to 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 are provided.

In addition, a kit and methods for identifying event TC1507 in a biological sample are provided.

In such kits, 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. Also disclosed are

~~The invention also relates to~~ 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. Also disclosed is

~~The invention also relates to~~ 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~~ disclosure 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~~ are also disclosed.

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The invention therefore provides an isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO: 21, 22, 24, 26 or 27.

The invention also provides a kit for identifying event TC1507 in a biological sample which detects a TC1506 specific region, said kit comprising a first primer which recognises SEQ ID NO: 21 or SEQ ID NO: 22, and a second primer which recognises SEQ ID NO: 25.

The invention also provides a DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

The invention also provides 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.

The invention also provides 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.

The invention also provides a 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

The invention also provides a method for identifying event TC1507 in a biological sample, comprising detecting a TC1507 specific region with a probe or first primer which specifically recognises a sequence within SEQ ID NO: 21 or SEQ ID NO: 22.

The invention also provides 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

The invention also provides an isolated DNA molecule comprising the amplicon produced by said method.

The invention also provides 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.

The invention also provides an isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

The invention also provides 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, or 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.

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

The invention also provides 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.

The invention also provides 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.

The invention also provides 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

The invention also provides 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.

The invention also provides 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.

→7d

The invention also provides 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, 49, 50, 51, 52, 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.

The invention also provides a method for identifying TC1507 in a biological sample which detects a TC1507 specific region within SEQ ID NO: 24.

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~~ the 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
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11201 GAACCGAGGG CAATCTGACG CAAGAAGGAA GAAGGAGCGG
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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.

SEQUENCE LISTING

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E. I. du Pont de Nemours and Company
Pioneer Hi-Bred International, Inc.

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<210> 10
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<400> 10
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ggtcatcgat gggaagggga a                                          11361

<210> 25
<211> 6186
<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
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aaggcc 6186

<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.

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tgcggctgtg gacagcgacg ctgtgacggc gactcggcga agccccaagc taccaagccc 600
ccaagtcccc atccatctct gcttctctgg tcatctcctt cccctggtcg atctgcaggc 660
gccagaccgg ccgaagcatc acgaaacgca ctaagacctc gaaggagtca aaccactcct 720
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<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.

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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
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>
<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
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 ccccccccccc 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
<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
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catgtcgtcg tcttcatcat cgtcttcatc atcgtcgtcg tcatcaggcg tctccggcga 360
cggctccctt gggagccccc 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
<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
tctcaaggcc 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

<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

<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
aaaccgcgga acacaagaac 20

Druckexemplar

~~MAIN REQUEST~~ - CLAIMS

1. An isolated DNA molecule comprising a nucleotide sequence identified as SEQ ID NO:21, 22, 24, 26 or 27.

2. A kit for identifying event TC1507 in a biological sample which detects a TC1507 specific region, said kit comprising a first primer which recognizes SEQ ID NO:21 or SEQ ID NO:22, and a second primer which recognizes SEQ ID NO:25.

3. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:1 and SEQ ID NO:2, respectively.

4. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:2 and SEQ ID NO:23, respectively.

5. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:3 and SEQ ID NO:5, respectively.

6. The kit of claim 2, wherein said at least first and second primers comprise the sequence of SEQ ID NO:4 and SEQ ID NO:5, respectively.

7. A DNA detection kit comprising at least one DNA molecule that is homologous or complementary to SEQ ID NO: 26 or 27, wherein the DNA molecule is specific for junction DNA of maize event TC1507 and is of a sufficient length of contiguous DNA polynucleotides to function in a DNA detection method.

8. 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.

9. 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.

10. 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

11. 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.

12. 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.

13. The method of claim 12, 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: 25.

14. The method of claim 13, wherein said first primer recognizes a sequence within SEQ ID NO:21 and said second primer recognizes a sequence within SEQ ID NO: 25.

15. The method of claim 12, wherein said first primer recognizes a sequence within SEQ ID NO:22 and a second primer recognizes a sequence within SEQ ID NO:25.

16. The method of claim 14, wherein said first and second primers comprise: the sequence of SEQ ID NO:2 and SEQ ID NO:1 respectively; or the sequence of SEQ ID NO:2 and SEQ ID NO:23 respectively.

17. The method of claim 15, wherein said first and second primers comprise: 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.

18. The method of claim 16, comprising amplifying a fragment of about 912 bp, about 844 bp, about 342 bp, or about 252 bp using a TC1507 PCR identification protocol.

19. 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, SEQ ID NO:2 and SEQ ID NO:23, SEQ ID NO:3 and SEQ ID NO:5, or 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.

20. An isolated DNA molecule comprising the amplicon produced by the method of claim 19.

21. 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.

22. An isolated DNA nucleotide primer sequence comprising a sequence selected from the group consisting of SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 or its complement.

23. 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, or 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.

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

25. 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.

26. 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.

27. 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: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

28. 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.

29. The pair of isolated DNA sequences of claim 28 wherein each is chosen from SEQ ID NO:26; or wherein each is chosen from SEQ ID NO:27.

30. 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.

31. The method of claim 30 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

32. 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, 49, 50, 51, 52, 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.

33. The DNA detection kit of claim 11 wherein each member of the 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, 49, 50, 51, 52, 54, 55, 56 and 57 and complements thereof.

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

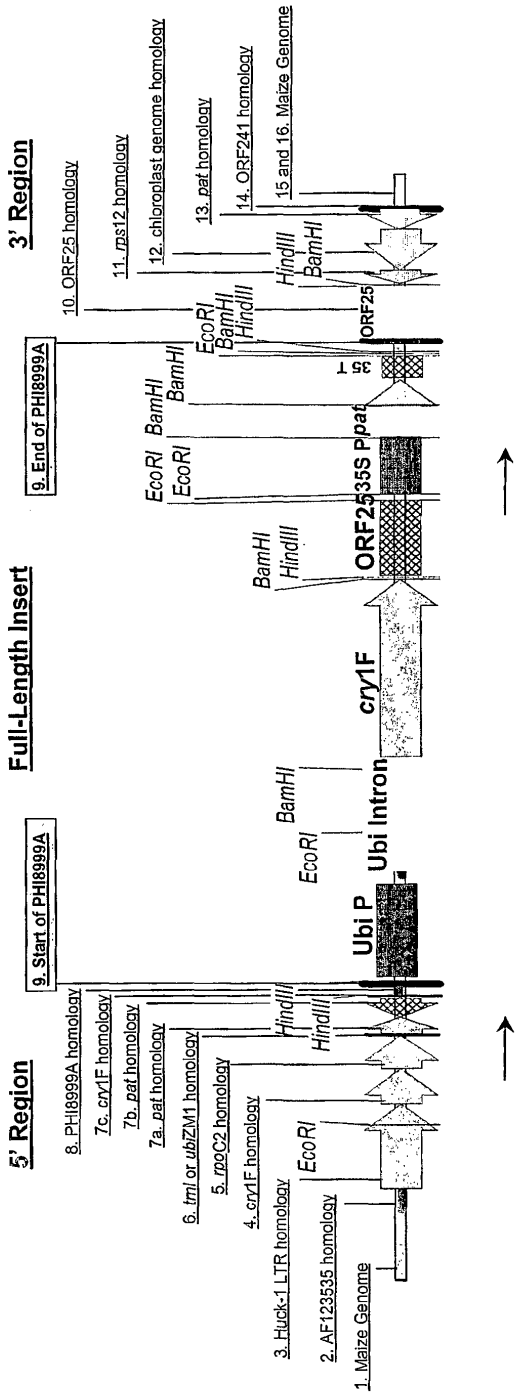

5' Region
Full-Length Insert
3' Region
1. Maize Genome
2. AF123535 homology
3. Huck-1 LTR homology
4. cry1F homology
5. rpoC2 homology
6. tml or ubiZM1 homology
7a. pat homology
7b. pat homology
7c. cry1F homology
8. PHI8999A homology
9. Start of PHI8999A
Ubi P
Ubi Intron
cry1F
ORF25
35S P
pat
35 T
9.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

FIG. 1

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Application No.: 04 751 097.9

IV.2. Patent classification

The classification indicated on the published patent application remains unchanged. It is as follows:

INV. C12Q1/68

IV.3. Title of the invention

The title indicated on the published patent application remains unchanged. It reads as follows:

"EVENT 1507" MAIS UND VERFAHREN FUR SEINE DETEKTION

CORN EVENT TC1507 AND METHODS FOR DETECTION THEREOF

MAIS TC1507 ET PROCEDES DE DETECTION DE CELUI-CI

IV.4. Documentation

STIN The file contains technical information submitted after the application was filed and not included in this specification.

CDOC The following documents not mentioned in the Search Report were cited during the Examination Procedure:

Anonymous: "OGM : pourquoi l'autorisation européenne de culture du maïs TC1507 ne va pas changer grand-chose dans l'immédiat | Atlantico. fr",
,
11 August 2014 (2014-08-11), XP055134128,
Retrieved from the Internet:
URL:http://www.atlantico.fr/decryptage/ogm-pourquoi-autorisation-europeenne-culture-mais-tc1507-ne-va-pas-changer-grand-chose-dans-immediat-marcel-kuntz-979208.html
[retrieved on 2014-08-11]

Anonymous: "TC1507",
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Retrieved from the Internet:
URL:http://www.sourcewatch.org/index.php/TC1507
[retrieved on 2014-08-11]

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Application No.: 04 751 097.9

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date 7 April 2015
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our ref 61.

Attn. Ms Claudia Weber

BY FACSIMILE

Dear Sirs

Name and address correction for E.I. du Pont de Nemours and Company

I refer to my recent telephone conversation with Claudia Weber in the client data registration department.

I confirm that the preferred format for the name and address of E.I du Pont de Nemours and Company is as follows:

E.I. du Pont de Nemours and Company
1007 Market Street
Wilmington
Delaware 19898
USA

As evidence of this, I attach extracts from the 2014 Form 10-K filed by E.I du Pont de Nemours and Company with the United States Securities and Exchange Commission. In particular, the second page of the attachment refers to the above as the name and address of the corporate headquarters.

I hope that the name and address of E.I du Pont de Nemours and Company as appearing on the EPO Register may now be standardised in the above preferred format. However, if there are any problems with this request, or if further evidence of the preferred format of the company name and/or the official address are required, please contact the undersigned.

Yours faithfully
Dehns

Annabel R. Beacham

Enc./nbs/arb

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Alexander J Pressdee BSc ○●
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● UK Chartered Patent Attorney, European Patent Attorney ○ UK Registered Trade Mark Attorney, European Trade Mark Attorney
Regulated by IPReg

Offices in London, Brighton, Oxford, Leeds, Manchester and Munich

E. I. du Pont de Nemours and Company

Form 10-K

Table of Contents

The terms "DuPont" or the "company" as used herein refer to E. I. du Pont de Nemours and Company and its consolidated subsidiaries, or to E. I. du Pont de Nemours and Company, as the context may indicate.

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Note on Incorporation by Reference

Information pertaining to certain Items in Part III of this report is incorporated by reference to portions of the company's definitive 2015 Annual Meeting Proxy Statement to be filed within 120 days after the end of the year covered by this Annual Report on Form 10-K, pursuant to Regulation 14A (the Proxy).

Duration: 07.04.2015 16:57:48 - 07.04.2015 16:58:23. This page 3 of 4 was completed at 07.04.2015 16:58:12
Received at the EPO on Apr 07, 2015 16:58:23. Page 3 of 4

Information for Investors

Corporate Headquarters
E. I. du Pont de Nemours and Company
1007 Market Street
Wilmington, DE 19898
Telephone: 302 774-1000
E-mail: http://www.dupont.com (click on Contact)

Stock Exchange Listings
DuPont common stock (Symbol DD) is listed on the New York Stock Exchange, Inc. (NYSE) and on certain foreign exchanges. Quarterly high and low market prices are shown in Item 5 of the Form 10-K.
DuPont preferred stock is listed on the New York Stock Exchange, Inc. (Symbol DDPrA for $3.50 series and Symbol DDPrB for $4.50 series).

Dividends
Holders of the company's common stock are entitled to receive dividends when they are declared by the Board of Directors. While it is not a guarantee of future conduct, the company has continuously paid a quarterly dividend since the fourth quarter 1904. Dividends on common stock and preferred stock are usually declared in January, April, July and October. When dividends on common stock are declared, they are usually paid mid March, June, September and December. Preferred dividends are paid on or about the 25th of January, April, July and October.

Shareholder Services
Inquiries from shareholders about stock accounts, transfers, certificates, dividends (including direct deposit and reinvestment), name or address changes and electronic delivery of proxy materials may be directed to DuPont's stock transfer agent:
Computershare Trust Company, N.A.
P.O. Box 30170
College Station, TX, 77842-3170
or call: in the United States and Canada
888 983-8766 (toll-free)
other locations-781 575-2724
for the hearing impaired-
TDD: 800 952-9245 (toll-free)
or visit Computershare's home page at
http://www.computershare.com/investor

Independent Registered Public Accounting Firm
PricewaterhouseCoopers LLP
Two Commerce Square, Suite 1700
2001 Market Street
Philadelphia, PA 19103

Investor Relations
Institutional investors and other representatives of financial institutions should contact:
E. I. du Pont de Nemours and Company
DuPont Investor Relations
1007 Market Street-D-11020
Wilmington, DE 19898
or call 302 774-4994

Bondholder Relations
E. I. du Pont de Nemours and Company
DuPont Finance
1007 Market Street-D-8028
Wilmington, DE 19898
or call 302 774-0564
or 302 774-2526

DuPont on the Internet
Financial results, news and other information about DuPont can be accessed from the company's website at **http://www.dupont.com**. This site includes important information on products and services, financial reports, news releases, environmental information and career opportunities. The company's periodic and current reports filed with the SEC are available on its website, free of charge, as soon as reasonably practicable after being filed.

Product Information/Referral
From the United States and Canada:
800 441-7515 (toll-free)
From other locations: 302 774-1000
On the Internet: http://www.dupont.com (click on Contact)

Printed Reports Available to Shareholders
The following company reports may be obtained, without charge:
1. 2014 Annual Report to the Securities and Exchange Commission, filed on Form 10-K;
2. Proxy Statement for 2015 Annual Meeting of Stockholders; and
3. Quarterly reports to the Securities and Exchange Commission, filed on Form 10-Q

Requests should be addressed to:
DuPont Corporate Customer Care
CRP - 735 (second floor)
974 Centre Road
Wilmington, DE 19805
or call:
From the United States and Canada: 800-441-7515 (toll free)
From other locations: 302-774-1000
On the Internet: http://www.dupont.com (click on Contact)

Services for Shareholders

Online Account Access
Registered shareholders may access their accounts and obtain online answers to stock transfer questions by signing up for Internet access by visiting http://www.computershare.com/investor. Shareholders have the option to request direct deposit of stock dividends, and electronic delivery of account statements and 1099-DIV tax forms.

Dividend Reinvestment Plan
An automatic dividend reinvestment plan is available to all registered shareholders. Common or preferred dividends can be automatically reinvested in DuPont common stock. Participants also may add cash for the purchase of additional shares. A detailed account statement is mailed after each investment. Your account can also be viewed over the Internet if you have Online Account Access (see above). To enroll in the plan, please contact Computershare (listed above).

Online Delivery of Proxy Materials
Shareholders may request their proxy materials electronically in 2015 by visiting **http://enrollicsdelivery.com/dd.**

Direct Deposit of Dividends
Registered shareholders who would like their dividends directly deposited in a U.S. bank account should contact Computershare (listed above).

04751097.9 - 1404 / 1620571 10.04.15

Client Database System (CDS) - clean up.

Application Nr.: 04751097.9

Following clean up action in CDS the entries concerning the **Applicant (02)** have been amended and are now as follows:

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
E. I. du Pont de Nemours and Company
1007 Market Street
Wilmington, DE 19898/US

Where appropriate, the European Patent Register at www.epo.org/register will be updated to show the amended details.

Questions about this communication? Contact Customer Services at www.epo.org/contact.

Europäisches Patentamt
European Patent Office
Office européen des brevets

Letter accompanying subsequently filed items

Representative:

Mr Andrew BENTHAM
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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	04751097.9
Applicant's or representative's reference	N96314-EP

	Description of document	Original file name	Assigned file name
1	Request for change of address	N96314-EP AXB KKT Change of Applicant address - Apr15.pdf	APPRCHOA-1.pdf

Signatures

Place:

Date: **30 April 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

30 April 2015

Dear Sirs

European Patent Application No. 04751097.9
DOW AGROSCIENCES LLC *et al*.
Our Ref: N96314-EP AXB/KKT/abj

Please update the address of PIONEER HI-BRED INTERNATIONAL, INC (i.e. the third Applicant) to:

7100 NW, 62nd Ave
PO Box 1014
Johnston
Iowa 50131-1014
United States of America

Yours faithfully

Electronically Signed
A BENTHAM

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • MUNICH

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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

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	3471156
Application number	EP04751097.9
Date of receipt	30 April 2015
Receiving Office	European Patent Office, The Hague
Your reference	N96314-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (1 p.) ep-sfd-request.xml APPRCHOA-1.pdf\N96314-EP AXB KKT Change of Applicant address - Apr15.pdf (1 p.)
Submitted by	CN=Andrew Bentham 23464
Method of submission	Online
Date and time receipt generated	30 April 2015, 19:05 (CEST)
Message Digest	A9:43:55:65:7E:DA:04:35:46:69:2A:81:B4:AE:2A:AF:5F:F1:14:5B

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

Letter accompanying subsequently filed items

Representative:

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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	04751097.9
Applicant's or representative's reference	N96314-EP

	Description of document	Original file name	Assigned file name
1	German translations of the claims	N96314-EP AXB KKT _DE translation of claims from PTI.pdf	CLMSTRAN-DE-1.pdf
2	French translations of the claims	N96314-EP AXB KKT _FR translation of claims from PTI.pdf	CLMSTRAN-FR-1.pdf
3	Reply to the communication under rule 71(3) EPC	N96314-EP AXB KKT EPG 71(3) EPO Response.pdf	IGRA-1.pdf

	Fees	Factor applied	Fee schedule	Amount to be paid
15-1	007 Fee for grant and printing (not more than 35 pages) or fee for grant including fee for publication	1	915.00	915.00
15-2	008 Additional printing fee for 36th and each subsequent page	41	15.00	615.00
	Total:		**EUR**	**1 530.00**

	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:	**18 May 2015**
Signed by:	**Kieron Grehan 23484**
Representative name:	**Kieron Grehan**
Capacity:	**(Representative)**

PATENTANSPRÜCHE

1. Isoliertes DNA-Molekül, umfassend eine Nukleotidsequenz identifiziert als SEQ ID NO: 21, 22, 24, 26 oder 27.

2. Kit für das Identifizieren von Ereignis TC1507 in einer biologischen Probe, die eine TC1507-spezifische Region erkennt, wobei das Kit einen ersten Primer umfasst, der SEQ ID NO: 21 oder SEQ ID NO: 22 erkennt, und einen zweiten Primer, der SEQ ID NO: 25 erkennt.

3. Kit nach Anspruch 2, wobei die mindestens ersten und zweiten Primer jeweils die Sequenz von SEQ ID NO: 1 und SEQ ID NO: 2 umfassen.

4. Kit nach Anspruch 2, wobei die mindestens ersten und zweiten Primer jeweils die Sequenz von SEQ ID NO: 2 und SEQ ID NO: 23 umfassen.

5. Kit nach Anspruch 2, wobei die mindestens ersten und zweiten Primer jeweils die Sequenz von SEQ ID NO: 3 und SEQ ID NO: 5 umfassen.

6. Kit nach Anspruch 2, wobei die mindestens ersten und zweiten Primer jeweils die Sequenz von SEQ ID NO: 4 und SEQ ID NO: 5 umfassen.

7. DNA-Nachweiskit, das mindestens ein DNA-Molekül umfasst, das homolog oder komplementär ist zu SEQ ID NO: 26 oder 27, wobei das DNA-Molekül für die Grenzschicht-DNA von Maisereignis TC1507 spezifisch und von einer ausreichenden Länge von zusammenhängenden DNA-Polynukleotiden ist, um in einem DNA-Nachweisverfahren zu funktionieren.

8. Kit für das Identifizieren von Ereignis TC1507 in einer biologischen Probe, wobei das Kit umfasst: eine spezifische Sonde, die eine Sequenz umfasst, die SEQ ID NO: 21 und SEQ ID NO: 25 zusammenhängend damit hybridisiert.

9. Kit für das Identifizieren von Ereignis TC1507 in einer biologischen Probe, wobei das Kit umfasst: eine spezifische Probe, die eine Sequenz umfasst, die mit SEQ ID NO: 22 und SEQ ID NO: 25 zusammenhängend damit hybridisiert.

10. DNA-Nachweiskit, das eine Polynukleotidsonde umfasst, die unter stringenten Hybridisierungsbedingungen mit einer oder mehreren DNA-Sequenzen hybridisiert, die ausgewählt sind aus der Gruppe bestehend aus SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 und 57 und Komplementen davon.

11. DNA-Nachweiskit, das ein Primerpaar umfasst, wobei jedes mindestens 10 Nukleotide von SEQ ID NO: 26 und SEQ ID NO: 27 umfasst, wobei sich jede auf entgegengesetzten Seiten einer Sequenzdiagnose für die TC1507-Ereigniseinfügung befindet.

12. Verfahren für das Identifizieren von Ereignis TC1507 in einer biologischen Probe, umfassend das Nachweisen einer TC1507-spezifischen Region mit einer Sonde oder einem ersten Primer, die bzw. der speziell eine Sequenz innerhalb von SEQ ID NO: 21 oder SEQ ID NO: 22 erkennt.

13. Verfahren nach Anspruch 12, weiter umfassend das Amplifizieren eines DNA-Fragments von einer Nukleinsäure, die in der biologischen Probe vorhanden ist, unter Verwendung einer Polymerasekettenreaktion mit mindestens zwei Primern, wobei der erste Primer eine Sequenz innerhalb von SEQ ID NO: 21 oder SEQ ID NO: 22 erkennt und ein zweiter Primer eine Sequenz innerhalb von SEQ ID NO: 25 erkennt.

14. Verfahren nach Anspruch 13, wobei der erste Primer eine Sequenz innerhalb von SEQ ID NO: 21 erkennt und der zweite Primer eine Sequenz innerhalb von SEQ ID NO: 25 erkennt.

15. Verfahren nach Anspruch 12, wobei der erste Primer eine Sequenz innerhalb von SEQ ID NO: 22 erkennt und

ein zweiter Primer eine Sequenz innerhalb von SEQ ID NO: 25 erkennt.

16. Verfahren nach Anspruch 14, wobei die ersten und zweiten Primer umfassen: jeweils die Sequenz von SEQ ID NO: 2 und SEQ ID NO: 1; oder jeweils die Sequenz von SEQ ID NO: 2 und SEQ ID NO: 23.

17. Verfahren nach Anspruch 15, wobei die ersten und zweiten Primer umfassen: jeweils die Sequenz von SEQ ID NO: 3 und SEQ ID NO: 5; oder jeweils die Sequenz von SEQ ID NO: 4 und SEQ ID NO: 5.

18. Verfahren nach Anspruch 16, umfassend das Amplifizieren eines Fragmentes von ungefähr 912 bp, ungefähr 844 bp, ungefähr 342 bp oder ungefähr 252 bp durch Verwenden eines TC1507-PCR-Identifikationsprotokolls.

19. Verfahren zum Nachweisen des Vorhandenseins von Maisereignis TC 1507 oder einem Abkömmling davon in einer biologischen Probe, umfassend:

(a) Extrahieren einer DNA-Probe von der biologischen Probe;

(b) Bereitstellen der DNA-Primermoleküle SEQ ID NO: 1 und SEQ ID NO: 2, SEQ ID NO: 2 und SEQ ID NO: 23, SEQ ID NO: 3 und SEQ ID NO: 5 oder SEQ ID NO: 4 und SEQ ID NO: 5;

(c) Bereitstellen von DNA-Amplifikationsreaktionsbedingungen;

(d) Ausführen der DNA-Amplifikationsreaktion, wodurch ein DNA-Amplikonmolekül erzeugt wird; und

(e) Nachweisen des DNA-Amplikonmoleküls, wobei das Nachweisen des DNA-Amplikonmoleküls in der DNA-Amplifikationsreaktion das Vorhandensein von Maisereignis TC1507 anzeigt.

20. Isoliertes DNA-Molekül, welches das Amplikon umfasst, das durch das Verfahren nach Anspruch 19 hergestellt wurde.

21. Verfahren zum Nachweisen des Vorhandenseins von DNA entsprechend des TC1507-Ereignisses in einer Probe, wobei das Verfahren umfasst:

(a) Kontaktieren der Probe, die Mais-DNA umfasst, mit einer Polynukleotidsonde, die unter stringenten Hybridisierungsbedingungen mit DNA von Maisereignis TC1507 hybridisiert und unter den stringenten Hybridisierungsbedingungen nicht mit einer Nicht-TC1507-Maispflanzen-DNA hybridisiert;

(b) die Probe und die Sonde stringenten Hybridisierungsbedingungen unterwerfen; und

(c) Nachweisen von Hybridisierung der Sonde zur DNA, wobei das Nachweisen der Hybridisierung das Vorhandensein von TC1507-Ereignis anzeigt.

22. Isolierte DNA-Nukleotidprimersequenz, die eine Sequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 und 57 oder ihrem Komplement.

23. Paar von DNA-Molekülen, umfassend: ein erstes DNA-Molekül und ein zweites DNA-Molekül, wobei die DNA-Moleküle von einer ausreichenden Länge von zusammenhängenden Nukleotiden von SEQ ID NO: 26 oder deren Komplement oder von SEQ ID NO: 27 oder deren Komplement sind, um als DNA-Primer oder Sondendiagnose für DNA zu fungieren, die von einer TC1507-Maispflanze oder einem Abkömmling davon extrahiert ist.

24. Isoliertes DNA-Molekül, umfassend eine Grenzschichtsequenz, die eine Sequenz umfasst, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 und 57 und Komplementen davon.

25. Verfahren zur Bestätigung von Saatgutreinheit, umfassend das Nachweisen einer TC1507-spezifischen Region mit einem spezifischen Primer oder einer Sonde, der bzw. die speziell eine Sequenz innerhalb von SEQ ID NO: 21 oder SEQ ID NO: 22 in einer Saatgutprobe erkennt.

26. Verfahren zum Screenen von Saatgut auf das Vorhandensein von Ereignis TC1507, umfassend das

Nachweisen einer TC1507-spezifischen Region mit einem spezifischen Primer oder einer Sonde, der bzw. die speziell eine Sequenz innerhalb von SEQ ID NO: 21 oder SEQ ID NO: 22 in einer Probe einer Saatgutpartie erkennt.

27. Isolierte DNA-Sequenz, umfassend eine Sequenz, die ausgewählt ist aus der Gruppe bestehend aus DNA, die mindestens eine Nukleotidsequenz aufweist, die ausgewählt ist aus der Gruppe bestehend aus SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 und 57 und Komplementen davon.

28. Paar von isolierten DNA-Sequenzen, wobei jedes mindestens zehn Nukleotide umfasst und die, wenn sie zusammen in einer DNA-Amplifikationsprozedur verwendet werden, eine Amplikondiagnose für Ereignis TC1507 herstellen.

29. Paar von isolierten DNA-Sequenzen nach Anspruch 28, wobei jede aus SEQ ID NO: 26 ausgewählt ist; oder wobei jede aus SEQ ID NO: 27 ausgewählt ist.

30. Verfahren zum Nachweisen des Vorhandenseins von TC1507-Ereigniseinfügung in Maisgewebe, umfassend:

(a) Auswählen eines Primerpaares, wobei jedes mindestens zehn Nukleotide von SEQ ID NO: 26 oder SEQ ID NO: 27 umfasst, wobei sich jedes Element des Paares auf

entgegengesetzten Seiten einer Sequenzdiagnose für die TC1507-Ereigniseinfügung befindet;

(b) Kontaktieren einer Probe des Maisgewebes mit dem Primerpaar;

(c) Ausführen von DNA-Amplifikation und Analysieren auf Amplika.

31. Verfahren nach Anspruch 30, wobei jedes Element des Primerpaars ausgewählt ist aus der Gruppe bestehend aus 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, 49, 50, 51, 52, 54, 55, 56 und 57 und Komplementen davon.

32. Verfahren zum Nachweisen des Vorhandenseins von TC 1507-Ereigniseinfügung im Maisgewebe, umfassend:

(a) Kontaktieren einer Probe des Maisgewebes mit einer Polynukleotidsonde, die unter stringenten Hybridisierungsbedingungen mit einer oder mehreren DNA-Sequenzen hybridisiert, die ausgewählt sind aus der Gruppe bestehend aus SEQ ID NO: 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 und 57 und Komplementen davon;

(b) die Probe und die Sonde stringenten Hybridisierungsbedingungen unterwerfen; und

(c) Analysieren auf Hybridisierung der Sonde.

33. DNA-Nachweiskit nach Anspruch 11, wobei jedes Element des Primerpaars ausgewählt ist aus der Gruppe

bestehend aus 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, 49, 50, 51, 52, 54, 55, 56 und 57 und Komplementen davon.

5

34. Verfahren zum Identifizieren von TC1507 in einer biologischen Probe, die eine TC1507-spezifische Region innerhalb von SEQ ID NO: 24 nachweist.

REVENDICATIONS

1. Molécule d'ADN isolée, comprenant une séquence nucléotidique identifiée par SEQ ID n° : 21, 22, 24, 26 ou 27.

2. Kit pour identifier un événement TC1507 dans un échantillon biologique qui détecte une région spécifique TC1507, ledit kit comprenant une première amorce qui reconnaît SEQ ID n° : 21 ou SEQ ID n° : 22, et une seconde amorce qui reconnaît SEQ ID n° : 25.

3. Kit selon la revendication 2, dans lequel lesdites au moins première et seconde amorces comprennent la séquence de SEQ ID n° : 1 et de SEQ ID n° : 2, respectivement.

4. Kit selon la revendication 2, dans lequel lesdites au moins première et seconde amorces comprennent la séquence de SEQ ID n° : 2 et de SEQ ID n° : 23, respectivement.

5. Kit selon la revendication 2, dans lequel lesdites au moins première et seconde amorces comprennent la séquence de SEQ ID n° : 3 et de SEQ ID n° : 5, respectivement.

6. Kit selon la revendication 2, dans lequel lesdites au moins première et seconde amorces comprennent la séquence de SEQ ID n° : 4 et de SEQ ID n° : 5, respectivement.

7. Kit de détection d'ADN comprenant au moins une molécule d'ADN qui est homologue ou complémentaire à SEQ ID n° : 26 ou 27, dans lequel la molécule d'ADN est spécifique pour l'ADN de jonction de l'événement TC1507 de maïs et est d'une longueur suffisante de polynucléotides d'ADN contigus pour fonctionner dans un procédé de détection d'ADN.

8. Kit pour identifier un événement TC1507 dans un échantillon biologique, ledit kit comprenant : une sonde spécifique comprenant une séquence qui s'hybride avec SEQ ID n° : 21 et SEQ ID n° : 25, contiguë à celles-ci.

9. Kit pour identifier un événement TC1507 dans un échantillon biologique, ledit kit comprenant : une sonde spécifique comprenant une séquence qui s'hybride avec SEQ ID n° : 22 et SEQ ID n° : 25, contiguë à celles-ci.

10. Kit de détection d'ADN comprenant une sonde polynucléotidique qui s'hybride dans des conditions d'hybridation strictes avec une ou plusieurs séquences d'ADN sélectionnées parmi le groupe constitué de SEQ ID n° : 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 et 57 et des compléments de celles-ci.

11. Kit de détection d'ADN comprenant une paire d'amorces comprenant chacune au moins 10 nucléotides de SEQ ID n° : 26 et de SEQ ID n° : 27, dans lequel chacune est sur des côtés opposés d'une séquence diagnostique pour l'insertion d'événement TC1507.

12. Procédé pour identifier un événement TC1507 dans un échantillon biologique, comprenant la détection d'une région spécifique TC1507 avec une sonde ou une première amorce qui reconnaît spécifiquement une séquence à l'intérieur de SEQ ID n° : 21 ou de SEQ ID n° : 22.

13. Procédé selon la revendication 12, comprenant en outre l'amplification d'un fragment d'ADN à partir d'un acide nucléique présent dans ledit échantillon biologique en utilisant une amplification en chaîne par polymérase avec au

moins deux amorces, dans lequel ladite première amorce reconnaît une séquence à l'intérieur de SEQ ID n° : 21 ou de SEQ ID n° : 22, et une seconde amorce reconnaît une séquence à l'intérieur de SEQ ID n° : 25.

14. Procédé selon la revendication 13, dans lequel ladite première amorce reconnaît une séquence à l'intérieur de SEQ ID n° : 21 et ladite seconde amorce reconnaît une séquence à l'intérieur de SEQ ID n° : 25.

15. Procédé selon la revendication 12, dans lequel ladite première amorce reconnaît une séquence à l'intérieur de SEQ ID n° : 22 et une seconde amorce reconnaît une séquence à l'intérieur de SEQ ID n° : 25.

16. Procédé selon la revendication 14, dans lequel lesdites première et seconde amorces comprennent : la séquence de SEQ ID n° : 2 et de SEQ ID n° : 1 respectivement ; ou la séquence de SEQ ID n° : 2 et de SEQ ID n° : 23 respectivement.

17. Procédé selon la revendication 15, dans lequel lesdites première et seconde amorces comprennent : la séquence de SEQ ID n° : 3 et de SEQ ID n° : 5 respectivement ; ou la séquence de SEQ ID n° : 4 et de SEQ ID n° : 5 respectivement.

18. Procédé selon la revendication 16, comprenant l'amplification d'un fragment d'environ 912 bp, d'environ 844 bp, d'environ 342 bp, ou d'environ 252 bp en utilisant un protocole d'identification d'ACP de TC1507.

19. Procédé de détection de la présence d'événement TC1507 de maïs ou d'une progéniture de celui-ci dans un échantillon biologique, comprenant :

(a) l'extraction d'un échantillon d'ADN à partir dudit échantillon biologique ;

(b) la fourniture de molécules d'amorce d'ADN SEQ ID n° : 1 et SEQ ID n° : 2, SEQ ID n° : 2 et SEQ ID n° : 23, SEQ ID n° : 3 et SEQ ID n° : 5, ou SEQ ID n° : 4 et SEQ ID n° : 5 ;

(c) la fourniture de conditions de réaction d'amplification d'ADN ;

(d) la réalisation de ladite réaction d'amplification d'ADN, produisant ainsi une molécule de produit d'amplification d'ADN ; et

(e) la détection de ladite molécule de produit d'amplification d'ADN, dans lequel la détection de ladite molécule de produit d'amplification d'ADN dans ladite réaction d'amplification d'ADN indique la présence d'événement TC1507 de maïs.

20. Molécule d'ADN isolée comprenant le produit d'amplification produit par le procédé selon la revendication 19.

21. Procédé de détection de la présence d'ADN correspondant à l'événement TC1507 dans un échantillon, le procédé comprenant :

(a) la mise en contact de l'échantillon comprenant de l'ADN de maïs avec une sonde polynucléotidique qui s'hybride dans des conditions d'hybridation strictes avec de l'ADN de l'événement TC1507 de maïs et ne s'hybride pas dans lesdites conditions d'hybridation strictes avec un ADN végétal de maïs non-TC1507 ;

(b) la soumission de l'échantillon et de la sonde à des conditions d'hybridation strictes ; et

(c) la détection de l'hybridation de la sonde avec l'ADN, dans lequel la détection de l'hybridation indique la présence

de l'événement TC1507.

22. Séquence d'amorce nucléotidique d'ADN isolée comprenant une séquence sélectionnées parmi le groupe constitué de SEQ ID n° : 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 et 57 ou son complément.

23. Paire de molécules d'ADN comprenant : une première molécule d'ADN et une seconde molécule d'ADN, dans laquelle les molécules d'ADN sont d'une longueur suffisante de nucléotides contigus de SEQ ID n° : 26 ou son complément, ou de SEQ ID n° : 27 ou son complément, pour servir d'amorces ou de sondes d'ADN diagnostiques pour l'ADN extrait d'une plante de maïs TC1507 ou d'une progéniture de celle-ci

24. Molécule d'ADN isolée comprenant une séquence de jonction comprenant une séquence sélectionnée parmi le groupe constitué de SEQ ID n° : 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 et 57 et des compléments de celle-ci.

25. Procédé pour confirmer la pureté des semences, comprenant la détection d'une région spécifique TC1507 avec une amorce ou une sonde spécifique qui reconnaît spécifiquement une séquence à l'intérieur de SEQ ID n° : 21 ou de SEQ ID n° : 22, dans un échantillon de semence.

26. Procédé pour dépister la présence d'un événement TC1507 dans des semences, comprenant la détection d'une région spécifique TC1507 avec une amorce ou une sonde spécifique qui reconnaît spécifiquement une séquence à l'intérieur de SEQ ID n° : 21 ou de SEQ ID n° : 22 dans un échantillon d'un lot de semences.

27. Séquence d'ADN isolée comprenant une séquence sélectionnée parmi le groupe constitué d'ADN possédant au moins une séquence nucléotidique sélectionnée parmi le groupe constitué de SEQ ID n° : 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 et 57 et des compléments de celle-ci.

28. Paire de séquences d'ADN isolées, comprenant chacune au moins dix nucléotides et qui, lorsqu'elles sont utilisées ensemble dans une procédure d'amplification d'ADN, produiront un produit d'amplification diagnostic pour l'événement TC1507.

29. Paire de séquences d'ADN isolées selon la revendication 28, dans laquelle chacune est choisie à partir de SEQ ID n° : 26 ; ou dans laquelle chacune est choisie à partir de SEQ ID n° : 27.

30. Procédé de détection de la présence de l'insertion d'événement TC1507 dans un tissu de maïs, comprenant :

(a) la sélection d'une paire d'amorces comprenant chacune au moins dix nucléotides à partir de SEQ ID n° : 26 ou de SEQ ID n° : 27, dans lequel chaque membre de la paire est sur des côtés opposés d'une séquence diagnostique pour ladite insertion d'événement TC1507 ;

(b) la mise en contact d'un échantillon dudit tissu de maïs avec ladite paire d'amorces ;

(c) la réalisation de l'amplification d'ADN et l'analyse pour découvrir un produit d'amplification.

31. Procédé selon la revendication 30, dans lequel chaque membre de la paire d'amorces est sélectionné parmi le groupe constitué de SEQ ID n° : 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 49, 50,

51, 52, 54, 55, 56 et 57 et des compléments de celui-ci.

32. Procédé de détection de la présence de l'insertion d'événement TC1507 dans un tissu de maïs, comprenant :

(a) la mise en contact d'un échantillon dudit tissu de maïs avec une sonde polynucléotidique qui s'hybride dans des conditions d'hybridation strictes avec une ou plusieurs séquences d'ADN sélectionnées parmi le groupe constitué de SEQ ID n° : 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 et 57 et des compléments de celles-ci ;

(b) la soumission dudit échantillon et de ladite sonde à des conditions d'hybridation strictes ; et

(c) l'analyse pour découvrir une hybridation de la sonde.

33. Kit de détection d'ADN selon la revendication 11 dans lequel chaque membre de la paire d'amorces est sélectionné parmi le groupe constitué de SEQ ID n° : 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 45, 46, 47, 49, 50, 51, 52, 54, 55, 56 et 57 et des compléments de celui-ci.

34. Procédé pour identifier TC1507 dans un échantillon biologique qui détecte une région spécifique TC1507 à l'intérieur de SEQ ID n° : 24.

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

European Patent Application No. 04751097.9
DOW AGROSCIENCES LLC *et al*
Our Ref: N96314-EP EPG

In reply to the Communication under Rule 71(3) EPC, we attach translations of the claims of this application into French and German.

We also request deduction of the fees necessary for grant from deposit account number 2805.0038 in the name of J A Kemp, namely:

EUR 915	Grant fee
EUR 615	Printing fee

We request that any shortfalls in the fees due are also deducted from the above-referenced deposit account.

Yours faithfully

Electronically Signed
K GREHAN
AUTHORISED REPRESENTATIVE

PATENT ATTORNEYS • TRADE MARK ATTORNEYS
LONDON • OXFORD • MUNICH

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

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European Patent Office
Office européen des brevets

Acknowledgement of receipt

We hereby acknowledge receipt of the following subsequently filed document(s):

Submission number	3504633
Application number	EP04751097.9
Date of receipt	18 May 2015
Receiving Office	European Patent Office, The Hague
Your reference	N96314-EP
Applicant	All applicants as on file
Documents submitted	package-data.xml epf1038.pdf (2 p.) CLMSTRAN-FR-1.pdf\N96314-EP AXB KKT _FR translation of claims from PTI.pdf (7 p.) ep-sfd-request.xml CLMSTRAN-DE-1.pdf\N96314-EP AXB KKT _DE translation of claims from PTI.pdf (9 p.) IGRA-1.pdf\N96314-EP AXB KKT EPG 71(3) EPO Response.pdf (1 p.)
Submitted by	CN=Kieron Grehan 23484
Method of submission	Online
Date and time receipt generated	18 May 2015, 17:09 (CEST)
Message Digest	67:49:4B:58:3E:80:B4:48:36:87:AD:FD:F3:CC:29:77:BA:42:4C:0C

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/

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European Patent Office
Office européen des brevets

European Patent Office
80298 MUNICH
GERMANY

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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
28.05.15

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Communication

concerning the registration of amendments relating to

☐ a transfer (R. 22 and 85 EPC)

☑ entries pertaining to the applicant / the proprietor (R. 143(1)(f) EPC)

As requested, the entries pertaining to the applicant of the above-mentioned European patent application / to the proprietor of the above-mentioned European patent have been amended to the following:

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
Dow AgroSciences, LLC
9330 Zionsville Road
Indianapolis, Indiana 46268/US

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
E. I. du Pont de Nemours and Company
1007 Market Street
Wilmington, DE 19898/US

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
PIONEER HI-BRED INTERNATIONAL, INC.
7100 NW 62nd Avenue
P.O. Box 1014
Johnston, IA 50131-1014/US

The registration of the changes has taken effect on 30.04.15.

In the case of a published application / a patent, the change will be recorded in the Register of European Patents and published in the European Patent Bulletin (Section I.12 / II.12).

Your attention is drawn to the fact that, in the case of the registration of a transfer, any automatic debit order only ceases to be effective from the date of its express revocation (cf. point 14(c) of the Arrangements for the automatic debiting procedure, see Supplementary publication 4 - OJ EPO 2014).

Receiving Section / For the Examining Division / For the Opposition Division / For the Legal Division *)

*) See note.

Note
This communication is issued by/for the department with whom responsibility lies. The Legal Division is responsible for the registration of transfers, changes of name (Articles 71, 72 and 74 EPC and Rules 22 and 85 EPC) as well as for the rectification of the designation of the inventor (Rule 21 EPC) (see Decision of the President of the EPO, OJ EPO 2013, 600). In all other cases, the Receiving Section, the Examining Division or the Opposition Division is responsible, depending on the stage in proceedings.

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Bentham, Andrew
J A Kemp
14 South Square
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London WC1R 5JJ
ROYAUME UNI

Date
05.06.15

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Decision to grant a European patent pursuant to Article 97(1) EPC

Following examination of European patent application No. 04751097.9 a European patent with the title and the supporting documents indicated in the communication pursuant to Rule 71(3) EPC dated 07.01.15 is hereby granted in respect of the designated Contracting States.

Patent No. : 1620571
Date of filing : 29.04.04
Priority claimed : 02.05.03/USP 467772

Designated Contracting States and Proprietor(s) : 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
Dow AgroSciences, LLC
9330 Zionsville Road
Indianapolis, Indiana 46268/US

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
E. I. du Pont de Nemours and Company
1007 Market Street
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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
PIONEER HI-BRED INTERNATIONAL, INC.
7100 NW 62nd Avenue
P.O. Box 1014
Johnston, IA 50131-1014/US

This decision will take effect on the date on which the European Patent Bulletin mentions the grant (Art. 97(3) EPC).

The mention of the grant will be published in European Patent Bulletin 15/27 of 01.07.15.

Registered letter
EPO Form 2006A 12.07 (28/05/15)

to EPO postal service: 29.05.15

Examining Division

Helliot B Jacques P Leber T

ANMERKUNG ZUR ENTSCHEIDUNG ÜBER DIE ERTEILUNG
EINES EUROPÄISCHEN PATENTS (EPA Form 2006)

1. **EPA Informationsbroschüre "Nationales Recht zum EPÜ"**
Diese Broschüre enthält nützliche Informationen zu den formalen Erfordernissen und den Handlungen, die vor den Patentbehörden der Vertragsstaaten vorzunehmen sind, um Rechte in diesen Staaten zu erlangen. Da diese Handlungen einem ständigen Wandel unterworfen sind, sollte immer nur die neueste Ausgabe der Broschüre benutzt werden. Nachträgliche Informationen werden im Amtsblatt veröffentlicht.

2. **Übersetzung der europäischen Patentschrift nach Artikel 65 (1) des Europäischen Patentübereinkommens**
Sie werden erneut darauf hingewiesen, dass bestimmte Vertragsstaaten nach Artikel 65 (1) EPÜ eine Übersetzung der europäischen Patentschrift verlangen; hierauf wird in der Mitteilung gemäß Regel 71 (5) EPÜ verwiesen. Die Nichteinreichung dieser Übersetzung kann zur Folge haben, dass das Patent in dem betreffenden Staat/in den betreffenden Staaten als von Anfang an nicht eingetreten gilt. Weitere Einzelheiten entnehmen Sie bitte der oben genannten Broschüre.

3. **Zahlung von Jahresgebühren für europäische Patente**
Nach Artikel 141 EPÜ können "nationale" Jahresgebühren für das europäische Patent für die Jahre erhoben werden, die an das Jahr anschließen, in dem der Hinweis auf die Erteilung des europäischen Patents im "Europäischen Patentblatt" bekanntgemacht wird. Weitere Einzelheiten entnehmen Sie bitte der oben genannten Broschüre.

NOTE RELATING TO THE DECISION TO GRANT A
EUROPEAN PATENT (EPO Form 2006)

1. **EPO Information Brochure "National law relating to the EPC"**
This brochure provides useful information regarding formal requirements and the steps to be taken before the patent authorities of the Contracting States in order to acquire rights in those states. Since the necessary steps are subject to change the latest edition of the brochure should always be used. Subsequent information is published In the Official Journal.

2. **Translation of the European patent application under Article 65(1) of the European Patent Convention**
Your attention is again drawn to the requirements regarding translation of the European patent specification laid down by a number of Contracting States under Article 65(1) EPC, to which reference is made in the communication under Rule 71(5) EPC. Failure to supply such translation(s) may result in the patent being deemed to be void "ab initio" in the State(s) in question. For further details you are recommended to consult the above-mentioned brochure.

3. **Payment of renewal fees for European patents**
Under Article 141 EPC "national" renewal fees in respect of a European patent may be imposed for the years which follow that in which the mention of the grant of the European patent is published in the "European Patent Bulletin". For further details you are recommended to consult the above-mentioned brochure.

REMARQUE RELATIVE A LA DECISION DE DELIVRANCE
D'UN BREVET EUROPEEN (OEB Form 2006)

1. **Brochure d'information de l'OEB "Droit national relatif à la CBE"**
Cette brochure fournit d'utiles renseignements sur les conditions de forme requises et sur les actes à accomplir auprès des offices de brevet des Etats contractants aux fins d'obtenir des droits dans les Etats contractants. Etant donné que les actes indispensables sont susceptibles de modifications, il serait bon de toujours consulter la dernière édition de la brochure. Toute information ultérieure est publiée au Journal Officiel.

2. **Traduction du fascicule du brevet européen en vertu de l'article 65(1) de la Convention sur le brevet européen**
Votre attention est de nouveau attirée sur l'obligation faite par certains Etats contractants, en vertu de l'article 65(1) CBE, da fournir une traduction du fascicule du brevet européen, à laquelle il est fait référence dans la notification établie conformément à la règle 71(5) CBE. Si la(les) traduction(s) n'est(ne sont) pas fournie(s), le brevet européen peut, dès l'origine, être réputé sans effet dans cet(ces) Etat(s). Pour plus de détails, nous vous renvoyons à la brochure susmentionnée.

3. **Paiement des taxes annuelles pour le brevet européen**
Conformément à l'article 141 CBE des taxes annuelles "nationales" dues au titre du brevet européen peuvent être perçues pour les années suivant celle au cours de laquelle la mention de la délivrance du brevet européen est publiée au "Bulletin européen des brevets. Pour plus de détails, nous vous renvoyons à la brochure susmentionnée.

Europäisches Patentamt
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GERMANY

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Bentham, Andrew
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ROYAUME UNI

Date
01.07.15

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Transmission of the certificate for a European patent pursuant to Rule 74 EPC

The certificate for a European patent is herewith transmitted.

The European patent specification can be downloaded from the EPO publication server **https://data.epo.org/publication-server/** (see OJ EPO 2005, 126).

Note:
A corrected title page of the European patent specification will be published, if the bibliographic data have been changed after completion of the technical preparations.

For the Examining Division



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80298 MUNICH
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Bentham, Andrew
J A Kemp
14 South Square
Gray's Inn
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ROYAUME UNI

Date
09.05.16

Reference	Application No./Patent No.
N.96314 AB	04751097.9 - 1404 / 1620571
Applicant/Proprietor	
Dow AgroSciences, LLC, et al	

Communication regarding the expiry of the time limit within which notice of opposition may be filed

You are hereby informed that on expiry of the nine-month time limit from the publication of the mention of the grant of European patent No. 1620571 no notice of opposition had reached the files.

The entry in the Register of European Patents will be automatically generated by the electronic data processing system.

For the Examining Division

Europäisches Patentamt • European Patent Office • Office européen des brevets

EPPU 02: 01.07.15 1404