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Radicación: 02049630 00000003

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Trámite : 002 PATENTES D 1 REGISTRO/ 444 REPUESTA

Dependencia: 2020 DIVISION DE NUEVAS CREACIONES

Señora Jefe de la
DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Comerc
E. S. D.

Trámite : 002

Evento : 001

Actuación: 444

Referencia : Expediente No. 02.049.630
Solicitante : MONSANTO TECHNOLOGY LLC
Asunto : Solicitud de patente para "PLANTA EVENTO DE
ALGODÓN MON15985 Y COMPOSICIONES Y METODOS
PARA DETECCIÓN DEL MISMO"
Fecha de solicitud : 11 de junio de 2002

1. Yo, Emilio FERRERO, obrando como apoderado del solicitante en el asunto antes identificado, me refiero al oficio número 13048, notificado por fijación en lista del 11 de julio de 2002.

2. Mediante el oficio número 13048 se puso en conocimiento del solicitante el informe que contiene las observaciones hechas por su Despacho sobre la presente solicitud de patente. Atiendo a las observaciones de la manera siguiente:

2.1. Cesión de los inventores. Presento junto con este escrito los documentos en que consta la cesión del derecho a la patente otorgado por los inventores al solicitante, debidamente legalizados, junto con sus respectivas traducciones oficiales.

2.2. Traducción de la solicitud. Me permito informarle que la traducción al español de la presente solicitud fue presentada junto con mi memorial número 02049630 00000001 radicado el 14 de junio de 2002.

2.3. Copia Certificada. Presento con este escrito la copia certificada de la solicitud de patente estadounidense número 60/297,406 del 11 de junio de 2001, junto con su respectiva

traducción de sus reivindicaciones y carátula, en la cual se basa la reivindicación de la prioridad.

3. Puesto que se han atendido a cabalidad las observaciones hechas por su Despacho sobre la presente solicitud de patente, atentamente solicito que se continúe su tramitación.

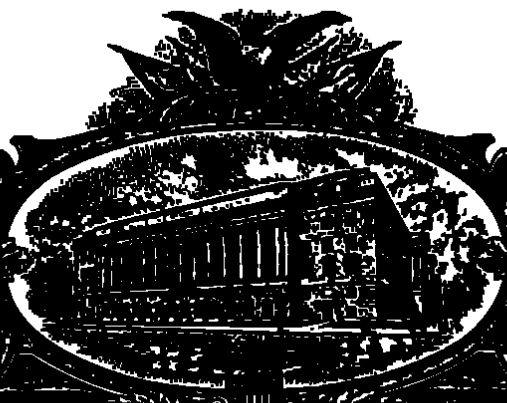
Bogotá, **15 AGO 2002**
Señora Jefe,



EMILIO FERRERO

T.P.A. No. 31.929

OUR COLO 12746-801-002-1
MRE/MRE/
ID-000009/WPC12746



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

June 11, 2002

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE UNDER 35 USC 111.

APPLICATION NUMBER: 60/297,406

FILING DATE: June 11, 2001



**By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS**


P. SWAIN
Certifying Officer

Please type a plus sign (+) inside this box Approved for use through 10/31/2002. OMB 0551-0001
U.S. Patent and Trademark Office, U.S. DEPARTMENT OF COMMERCE

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.63(e).

INVENTOR(S)

Given Name (first and middle (if any))	Family Name or Surname	Residence (City and either State or Foreign Country)
Scott	Huber	Grave Coeur, Missouri

☒ Additional inventors are being named on the 1 separately numbered sheets attached hereto**TITLE OF THE INVENTION (250 characters max)**

COTTON EVENT MONITORING AND COMPOSITIONS AND METHODS FOR DETECTION THEREOF

Direct all correspondence to:

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27161

PATENT TRIANGLE OFFICE

Address

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State

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Fax

ENCLOSED APPLICATION PARTS (check all that apply)☒ Specification Number of Pages

44

☐ CD(s), Number☒ Drawing(s) Number of Sheets

1

☒ Other (specify)

Sequence Listing (15 pages)

☐ Application Data Sheet. See 37 CFR 1.78**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 fee.☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number:

13-4125

☐ Payment by credit card. Form PTO-2036 is attached.FILING FEE
AMOUNT (\$)

\$150.00

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

TYPED or PRINTED NAME Timothy K. Ball

TELEPHONE (636) 737-7387

Date

6/11/01

REGISTRATION NO.

(if appropriate)

Docket Number:

42,287

SB-21(52211)

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.61. 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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PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SS/18 (9-00)
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Docket Number		38-21(52211)	Type a plus sign (+) inside this box →	+
INVENTOR(S)/APPLICANT(S)				
Given Name (first and middle (if any))	Family or Surname	Residence (City and either State or Foreign Country)		
James K.	Roberts	Chesterfield, Missouri		
Zachary	Shappley	Wildwood, Missouri		

Number 1 of 1

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60297406-061101

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**FEE TRANSMITTAL
for FY 2001**

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT (\$)**150.00**

Complete if Known

Application Number	Unknown
Filing Date	June 11, 2001
First Named Inventor	Scott Huber
Examiner Name	Unknown
Group Art Unit	Unknown
Attorney Docket No.	38-21(52211)

METHOD OF PAYMENT

- 1.
- ☒
- The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:

Deposit
Account Number
13-4125Deposit
Account Name
Monsanto Company

- ☒
- Charge Any Additional Fee Required
-
- Under 37 CFR 1.16 and 1.17
-
- ☐
- Applicant claims small entity status,
-
- see 37 CFR 1.37

- 2.
- ☐
- Payment Enclosed:

☐ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity	Small Entity	Fee Code	Fee Code	Fee Description	Fee Paid
101	710	201	355	Utility filing fee	
108	330	205	180	Design filing fee	
107	480	207	245	Plant filing fee	
106	710	206	355	Reissue filing fee	
114	180	214	75	Provisional filing fee	150.00

SUBTOTAL (1) (\$)**150.00**

2. EXTRA CLAIM FEES

Total Claims	Extra Claims	Fee from	Fee Paid
Independent	-30*-	X	
Multiple Dependent	-3*-	X	

Large Entity	Small Entity	Fee Code	Fee Code	Fee Description	Fee Paid
103	15	203	9	Claims in excess of 20	
102	80	202	40	Independent claims in excess of 3	
104	270	204	135	Multiple dependent claim, if not paid	
105	80	205	40	** Release independent claims over original patent	
110	15	210	9	** Release claims in excess of 20 and over original patent	

SUBTOTAL (2) (\$)**0**

**or number previously paid, if greater; For Releases, see above

FEE CALCULATION (continued)

Large Entity	Small Entity	Fee Code	Fee Code	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	80	227	25	Surcharge - late provisional filing fee or cover sheet	
136	130	136	130	Non-English specification	
147	2,620	147	2,620	For filing a request for an <i>ex parte</i> reexamination	
112	820*	112	820*	Requesting publication of BIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of BIR after Examiner action	
116	110	216	55	Extension for reply within first month	
116	360	216	185	Extension for reply within second month	
117	600	217	445	Extension for reply within third month	
118	1,280	218	895	Extension for reply within fourth month	
128	1,880	228	945	Extension for reply within 5th month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
131	270	231	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,240	241	620	Petition to revive - unintentional	
143	1,240	243	620	Utility issue fee (per release)	
143	440	243	220	Design issue fee	
144	800	244	300	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	130	123	130	Petitions related to provisional applications	
125	180	125	180	Submission of Information Disclosure Sheet	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	710	246	355	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	710	249	355	For each additional invention to be submitted (37 CFR § 1.129(b))	
178	710	278	355	Request for Continued Examination (RCE)	
189	900	189	900	Request for expedited examination of a design application	

Other fee (specify):

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$)**0**

SUBMITTED BY

Name (Print/Type)

Timothy K. Ball

Registration No.

42,257

Complete if applicable

Telephone

(838) 737-7387

Signature

Date

6-11-01

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60297406-061101

CERTIFICATE UNDER 37 C.F.R. §1.10

Timothy K. Ball

1

1

1

11

2

TRANSMITTAL LETTER

☒ **Provisional Application for Patent Cover Sheet;**
☒ **Fee Transmittal for Year 2001;**
☒ **Provisional Patent Application;**
☒ **Paper Copy of Sequence Listing;**
☒ **If an extension or an additional extension of time is required, but is not enclosed, please consider this a conditional petition therefor and charge Deposit Account 13-4125 accordingly.**
☒ **Please charge the \$150.00 fee to Deposit Account 13-4125.**
☒ **Please charge any deficiencies or credit any overpayment to Deposit Account 13-4125. A duplicate copy of this sheet is attached.**


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IN THE UNITED STATES PATENT OFFICE

A

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PROVISIONAL APPLICATION

FOR

10

COTTON EVENT MON15985 AND COMPOSITIONS AND METHODS
FOR DETECTION THEREOF

INVENTORS:

15

SCOTT HUBER
JAMES K. ROBERTS
&
ZACHARY SHAPPLEY

20

60297405-061101

FIELD OF THE INVENTION

This invention is related to the field of plant molecular biology, and in particular to plant insect protection and plant breeding, and is directed to a novel transformation event of cotton plants, *Gossypium hirsutum*, comprising at least one polynucleotide sequence inserted into a specific site within the genome of a cotton cell, said sequence encoding a Cry2Ab lepidopteran insect inhibitory protein. Additionally, the invention is related to cotton plants derived from transformation events designated as MON15985, MON15985X, and MON531, and to assays for detecting the presence of these cotton events, their nucleic acid complement, or protein produced from their respective event specific DNA, in a sample.

BACKGROUND OF THE INVENTION

This invention relates to the Lepidopteran resistant cotton plant (*Gossypium hirsutum*) event MON531 expressing a chimeric form of a Cry1A insect inhibitory protein and derivative events thereof comprising MON15985 and MON15985X, each of which expresses a Cry2A insect inhibitory protein. Additionally, the invention relates to the plants as designated, progeny and plant parts thereof, and to methods for the detection of the transgene or genomic insertion regions in said cotton line events and in progeny and plant parts thereof, as well as to methods for detecting the products of the transgenes inserted into the cotton genome within these specific cotton lines.

Cotton is a commercially valuable textile and is best known as a fibrous composition produced by cotton plants of, or related to the genus and species *Gossypium hirsutum*. Cotton plants are susceptible to insect infestation in all areas of the world in which the plants are cultivated. Recombinant DNA technology has been applied to cells of the cotton plant for about a decade, and cotton plants which exhibit improved characteristics as a result of the insertion of heterologous DNA sequences have been produced using recombinant DNA technology since about the early 1990's. Some of the improvements exhibited by recombinant cotton plants consist of herbicide tolerance, improved fiber characteristics, and resistance to insect infestation.

The first recombinant cotton plants protected from *Lepidopteran* insect infestation were produced, approved by regulatory agencies for commercial distribution, and subsequently commercialized in 1996. These cotton plants contained a DNA sequence encoding a chimeric

Cry1A lepidopteran insect inhibitory protein. This particular trait, along with an adjacent linked DNA sequence encoding a selectable marker, has been transferred by conventional breeding into a number of cotton varieties, each of which are particularly suited for enhanced production of cotton in diversified geographic locations throughout the world. These recombinant varieties have enjoyed a tremendous commercial success for a number of reasons. One reason is that yields per acre of cotton production have on average improved dramatically because of reduced insect infestation as a result of the presence of the insect inhibitory protein present within each cell of the cotton plant. Another principle reason for the commercial success has been the reduced labor and expense due to the reduction in over the top applications of chemical pesticides required to protect the crop from insect infestation. In addition, the reduction in chemical pesticide applications improves the overall health of the environment by avoiding the annihilation of insects or arachnids and other species which present no threat to the crop in the field, reduces the load of chemical pesticide toxins applied to the environment, and allows the farmer to avoid the risk associated with the potentially harmful effects of exposure to chemical pesticides.

A caveat, however, to the introduction of an insect resistance trait into a crop species is the concern related to the development of insect resistance. The management of the development of resistance, known as insect resistance management, has been a principle concern of the agricultural community. Not long after the introduction of chemical pesticide applications to crop fields in the late 1800's and early 1900's, it was noted that insect species adapted rapidly to the pesticides in their environment so that individuals which survived a particular dose of chemical pesticide would give rise to a rapid increase in populations of insects which were tolerant to the insecticide chemical. The only means of managing the development of races of insect pests which exhibited resistance to chemical pesticide applications was to apply high levels of two or more chemical insecticides simultaneously, each insecticide exerting its activity through a mode of action different from the other pesticide(s) in the composition, whenever insect infestation was observed to be problematic during a growing season. Theoretically, a population of insect pest species would not be able to develop resistance to both chemical pesticides in a mixture and so the combination would result in the control not only of insect infestation but also of the problem related to the development of populations of insects resistant to either of the chemical pesticides. Yet, utilizing the chemical

insecticide applications in this manner resulted in a greater sterilization of the crop fields because virtually all arachnid and insect species, i.e., pest species and species beneficial to the field or otherwise, were exterminated.

A more practical solution has been to combine two or more insect inhibitory proteins toxic to the same insect species together in a single plant to achieve a means similar to the combinatorial chemistries described above, for combating the development of pests resistant to insect inhibitory proteins present in a particular variety of recombinant insect resistant cotton. While it may be seen as obvious in view of the chemical arts to provide a combination of insect inhibitory proteins for resistance management purposes, both of which are toxic to the same insect pest species feeding on a particular plant, in reality the development of a plant meeting these characteristics is problematic, and requires a great deal of resources, technical ability, and trial and error experimentation in order to obtain a single recombinant plant transformation event which results in a morphologically normal recombinant plant exhibiting the desired combination of insect inhibitory proteins produced in sufficient levels and at appropriate times during the plant growing season and in the tissues upon which target pest species feed.

Thus, there exists a need for the development and characterization of cotton plants exhibiting the characteristics of enhanced insect resistance as a result of the presence of two or more insect inhibitory proteins produced from DNA sequences incorporated into the genome of the plant cells. Furthermore, it would be desirable for the insect inhibitory traits to segregate independent of one another, to not cause any adverse effects upon the physiology and metabolism of the plant, and to have little if any effect upon the yield or quality of the fiber produced from said plant.

Cotton event MON531, which contains a cassette encoding a chimeric Cry1A protein and a selectable marker, was transformed with a different expression cassette encoding a Cry2Ab insect control protein. Cotton plants expressing both the chimeric Cry1A and Cry2Ab proteins have improved insecticidal efficacy, an expanded range of insect control, and provide enhanced Insect Resistance Management options to the grower.

The expression of foreign genes in plants is known to be influenced by their chromosomal position, perhaps due to chromatin structure (e.g., heterochromatin) or the proximity of transcriptional regulation elements (e.g., enhancers) close to the integration site (Weising *et al.*, 1988, "Foreign Genes in Plants," *Ann. Rev. Genet.* 22:421-477). It is likely

that many insertional events create genetic instability, or for any number of reasons create problems in plants derived from such events including but not limited to chlorosis, stunting, and infertility. In any event, and at least for these reasons, it is often necessary to screen a large number of transformation events in order to identify a morphologically normal 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 wide variations in levels of expression of an introduced gene among individually selected 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 for a single event that has desired transgene expression levels and patterns for commercial purposes. An event that exhibits the 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 are expected to 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 thermal amplification (PCR™) or DNA hybridization using nucleic acid probes. Typically, for the sake of simplicity and uniformity of reagents and methodologies for use in detecting a particular DNA construct that has been used for transforming various plant varieties, these detection methods generally focus on frequently used genetic elements, such as promoters, terminators, marker genes, etc., because

for many DNA constructs, the coding sequence region is interchangeable. As a result, such methods may not be useful for discriminating between separate events produced as a result of using constructs which differ only with reference to as little as the coding sequence. In addition, such methods may not be useful for discriminating between different events, particularly those produced using the same DNA construct unless the sequence of chromosomal DNA adjacent to the inserted DNA ("flanking DNA") is known. An event-specific thermal amplification (PCRTM) assay is discussed, for example, by Windels et al. (*Med. Fac. Landbouww. Univ. Gent* 64/5b:459-462, 1999), who identified glyphosate tolerant soybean event 40-3-2 using a thermal amplification primer set spanning the junction between the insert and flanking DNA. Specifically, one primer included sequence from within the insert and a second primer included sequence from flanking DNA.

SUMMARY OF THE INVENTION

According to one aspect of the invention, compositions and methods are provided for detecting the presence of various transgene/genomic insertion regions from cotton plants designated as cotton event MON531, and cotton event MON15985 or a segregant variety derived from MON15985 designated as MON15985X.

DNA sequences are provided that comprise at least one junction sequence of MON531 selected from the group identified as SEQ ID NO:1 [5'GCGTT TCTGG TTATA ATATA3'] and SEQ ID NO:2 [5'CTTCT CTGCT AAGTG GGTCA3'] and complements thereof; wherein a junction sequence spans the point at which heterologous DNA inserted into the genome is linked to DNA from the cotton cell genome flanking the insertion point, the junction sequence(s) being diagnostic for the event.

Another aspect of the invention are various combinations of the DNA sequences that comprise at least 11 or more contiguous nucleotides of the DNA sequence of SEQ ID NO:3

	(agtaacacaa acaaaagcat catatgctga ttatcttatt catagatgga gctcaagtca	60
	tagttaata gcccgatact ttctcgcta actatgagat attacagcat acattttagt	120
	actacatact tattoagtaa aaagccctca aattgaaga caaaggaagc gatccccgg	180
30	taccgagtc gaattcaggt ctctagatct cattattctt caatcaagag aagctccacg	240
	ctgtccacga tgaaggtctc ctgggtttaa ccgattctga tccacattt gtgggtctca	300
	ggaaagtact caagtcctt ggtaacatag caactgga gtggtgtgta gtccctgtaa	360
	cctctgtta actgcaagc gttctcagc ctgcattctg tctaggattt ctctctgtac	420
	acggagcat agtcagcag aacggaagga gttcgttgt aacctctgtt acggttagtg	480

tagggaacctc cgtactcttc ctgattccca gtgtagtctg tgcaggtaac ggtgttgttg 38-21(52211)
540
ggatagattt otccotcgac gcagttggag aacttaagct cgtcgtgtgt gttctcgatc 600
tcgtggatgg tcaagtaacc ctcaacgtat cctcccttgt aagcgttcar accgagatg 660
tagctctac ctggacagac tctaacctct tgggaacctt cagcttccca ctcaaggaca 720
5 accaggaagg aacgtgtatt gttctgttcc tccacgtcca catgaccttt cactttccag 780
cagctgaggc cattgttga gtcacgttc ttgatgactt ttctggcctc gtacaaggag 840
aatgcgtta agtacgtcc ctcaagttcc tggagatgg cagcgttcc accagggatc 900
accggaacct caggcaagta agctctacga atgctgtgca cactttgtc tgcggcgtgg 960
atcatggcga tcttggcttc ggtctgcaac tgatctatt gggagttcac gaacaaagca 1020
10 tdcacggact ctttggctc cttgtaaag atgttagttt cccattcgag tttctcact
ttgtccctcc actctcttc tget 1104);

SEQ ID NO:4

laagtggtca gtgggtggc attggtttt aatttcaag cgttttctcg catatacttt 60
tatgattggg gtttctctt ggtgtacatc aatgaccttc cctattgat tgtctgaatt 120
15 ttcattcttt aatcggcta aaaaacaact atgaaaaa gagtaggact actcatcgt
accggggagg gggggggaa tggaggaat agaataggg actcatttat atatatata 240
accaagtatg ctattggtt atcaggtgg aggaattca atgttcaatt ctgggttcca 300
aatttgcct 309);

SEQ ID NO:5

ccctcaant gtcaatagg gtgcacctca tctgtcaga ctctgacct caagtgtcaa 60
20 ggtcgcgccc cctcatctgt cagtgtcgc ggcctcaag tgcataacc gcagggaact 120
tatccacagg ctgtccaca tcatctgtg gaactcgc taaaacagg cgttttgc 180
gatttgcgag gctggccagc tccactcgc ggtcgaaat cagactgca cctcatctgt 240
caacgcgcgc cgggtgagt cggccctca agtgtcaag tccgacctc atctgtcagt 300
25 gagggtcaag ttttcgcga ggtatccca acgcggcgg cgtcgtgtc tgcacacgg 360
cttcacggc gttcttg 378);

and SEQ ID NO:6

ttataatata cactatata atttatcct gtatatctt gaaagaaac atcagaggc 60
attgacctt cactttttt aaaaattt tctctgata gagaaagaa agaaagaaa 120
30 agaaatat tagtgcctt tcaatggtg aaaaacaaa aaaaatctc ttaatatata 180
aacaatatgt caaacgtct gacagctct g 211);

each respectively designated otherwise as the sequence of the insert at the 5' end; the cotton
genome sequence flanking the 5' end of the inserted sequence; the sequence of the insert at
the 3' end; and the cotton genome sequence flanking the 3' end of the inserted sequence and
35 complements thereof for use as DNA primers in DNA amplification methods. Appropriate
amplicons produced using such primers are diagnostic for cotton event MON531. Examples
of such primers are

SEQ ID NO:7 [gccaatgact cgtgattgtt ctctgc], and

SEQ ID NO:8 [gatttgcgag gctggccagc tccacg].

Sequence from which amplification primers can be selected are at least

SEQ ID NO:9

[cccccaaat gtcaataggt ggcgccctca tctgtcagca ctctgccct caagtgtcaa 60
 5 ggatcgccgc cctcatctgt cagtagtcgc gccccccaag tgtcaatacc gcagggcaat 120
 tatcccagc cttgtccaca tcatctgtgg gaactcgcg taaatcagc cgttttcgcc 180
 10 gatttgcag gctggccagc tccacgtcgc cggccgaat cgagcctgc cctcatctgt 240
 caacgcgcg cgggtgagt cggccctca agtgtcaacg tccgccctc atctgtcagt 300
 gagggccaag ttttcgcga ggtatccaca acgcggcgg cgcgggtgtc tgcacacgg 360
 15 cttcgacggc gtttctggtt ataataaca catatataat ttatcactgt atattcttgc 420
 agagacaat cagcggcat tggccctcc attttttta aaaaaatttg atctgataga 480
 20 gaagaagag aagaaaaag aagaatatta gtgaccttc atgggtgaa aatcaaaaa 540
 aatctcatt taatgataa caaatgtca aacagtctga cagctcctg 589],

and

SEQ ID NO:10

25 [agtacacaaa acaaaagcat catatgctga tttatttatt catagatgga gtcgaagtca 60
 tagttaata gccgatact ttctcgcgc actatgagct attacagcat acattttagt 120
 30 actacatact tattcagtaa aaagccctca aaattgaaga caaaggacgg gatccccgg 180
 taccgagctc gaattcaggc ctctagatct cattattcct ccatcaagag aagctccacg 240
 ctgtccacga tgaaggttc ctgggtttca ccgatctcga tccacacttt gtcggtctca 300
 35 ggaagtact caagctcctt ggtaacatag ccaactgga gtggtgtgta gtcctctgta 360
 cctctgttga actcgcaagg gttctcagct ctgccatctg tgtaggattt ctctctgtac 420
 acggagcat agtcagcag aaagggaagg gttctgtgt aaectctgtt acggctagt 480
 40 taggcacctc cgtactcttc ctgattcaca gtgtagtcgt tgcagtaac gttgttgtt 540
 ggatagattt ctctctgac gcagtggag aacttaagct cgtcgtgtt gttctcgtc 600
 45 tcgtggatgg tcacgcaacc ctcaacctat cctccttgt aagcgtcac acggagaatg 660
 tagcctctac ctggacagac tcaacctct tgggacatt cagcttccca ctcaaggaca 720
 accaggacgg aacgttgatt gttctgttcc tccaagtcca catgacctt cacattccag 780
 50 cagctgaggg cattgttga gtcaccttc ttgatgacgt ttttggcacc gtacaaggag 840
 aatgggttaa agatacgtc ctcaagttcc tcgaagatgg cagcgttcc accagggato 900
 55 acggacaact caggcaagta agcttcacga atgctgtgca cagctttgtc tgcggcgtg 960
 atcatggcga cgttgggtgc ggtttgcac tgatcatatt gggagttcac gaacaagca 1020
 tccacggact ctttggctc cttgtaaacg atgttagttt cccattcgag tttctcagct 1080

ttgtccctcc acttcttctc tgcctagtggt gtcagtggtg tggcattggt ctttaatttc 1140
 5 aacgggtttt gtcgcatata cttttatgat tgggttttct ctttgggtga catcaatgac 1200
 ctccctatt gcattgtctg aattttcacc tcttaaatcg gctaaaaaac aactatgaa 1260
 aaagagtag gctactcat ccgtaccggg gagggggggg ggaatggagg aataganta 1320
 10 ggggactcat ttatatatat ataaccag tatgttattg gttgatcgag tgggaaggaa 1380
 ttcattgttc aattctgggt ttgaacttg c 1411] .

The amplicons produced by said DNA primers are also an aspect of the invention.

15 According to another aspect of the invention, methods of detecting the presence of
 DNA corresponding to the cotton event MON531 in a sample are provided. Such methods
 comprise: (a) contacting the sample comprising DNA with a primer set that, when used in a
 nucleic acid amplification reaction with DNA from cotton event MON531, produces an
 amplicon that is diagnostic for cotton event MON531; (b) performing a nucleic acid
 20 amplification reaction, thereby producing the amplicon; and (c) detecting the amplicon.

According to another aspect of the invention, methods of detecting the presence of a
 DNA corresponding to the MON531 event in a sample, such methods comprising: (a)
 contacting the sample comprising DNA with a probe that hybridizes under stringent
 hybridization conditions with DNA from cotton event MON531 and does not hybridize under
 25 stringent hybridization conditions with a control cotton plant DNA, i.e., using other than
 MON531 DNA; (b) subjecting the sample and probe to stringent hybridization conditions;
 and (c) detecting hybridization of the probe to the DNA.

According to one aspect of the invention, DNA sequences are provided that comprise
 at least one junction sequence of cotton event MONMON15985 or MON15985X selected
 30 from the group consisting of SEQ ID NO:14 [tgaaatgggt ttattcgat], SEQ ID NO:15
 [acgaaggagt tcttttaggc], SEQ ID NO: 16 [atatctctat aaagggtaat], SEQ ID NO: 17
 [ctagtggatc tgcactgaaa], and SEQ ID NO: 18 [caagtaggg gttgttggt], and
 complements thereof. These sequences correspond to diagnostic features within the
 chromosome of plants comprising MON15985 or MON15985X genetic material. SEQ ID
 35 NO:14 corresponds to a 5' end diagnostic junction sequence between native and contiguous
 cotton genomic 5' end DNA flanking the inserted DNA, which is linked to the 5' end of a
 chloroplast related DNA sequence near the 5' end of the inserted DNA. SEQ ID NO:15
 corresponds to a 5' end diagnostic junction sequence between the 3' end of chloroplast related

DNA sequence linked to the 5' end of the 5' end cotton genomic remnant sequence positioned immediately 5' to the inserted 5' DNA end. SEQ ID NO:16 corresponds to a 5' end diagnostic junction sequence between the 3' end of the 5' end cotton genomic remnant DNA sequence and the 5' end of the inserted DNA sequence (i.e., the 5' end of the inserted DNA sequence is represented by a 35S promoter sequence driving expression of GUS from within the inserted DNA). SEQ ID NO:17 corresponds to a 3' end diagnostic junction sequence between the 3' end of the inserted DNA and the 5' end of the 3' end cotton genomic remnant DNA sequence which is positioned between the inserted DNA and the cotton genomic DNA flanking the 3' end of the inserted DNA. SEQ ID NO:18 corresponds to a 3' end diagnostic junction sequence between the 3' end of the 3' end cotton genomic remnant DNA sequence and the cotton genomic DNA sequence flanking the 3' end of the inserted DNA.

It should be noted that the identification or detection of a sequence diagnostic for cotton event MON15985X is insufficient to be diagnostic for cotton event MON15985. MON15985X is a variant of MON15985 containing a complement of genetic material substantially equivalent to MON15985 but which has been segregated away from the diagnostic sequences comprising cotton event MON531 which are also present in MON15985. Therefore, detection or identification of sequences which are diagnostic for cotton event MON15985X is insufficient to conclude that cotton event MON15985 DNA has been identified. One or more cotton event MON531 sequences must also be identified or detected in order for a detection or identification of sequences corresponding to cotton event MON15985X to also be diagnostic for cotton event MON15985.

A junction sequence herein spans the point at which heterologous DNA inserted into the genome is linked to DNA from the cotton cell genome flanking the insertion point, the identification or detection of one or more junction sequences in a plants' genetic material being sufficient to be diagnostic for the event. Included are DNA sequences that comprise at least 10 or more (e.g., 15, 25, 50) nucleotides of insert sequence from cotton event MON15985 or MON15985X and similar length of flanking DNA from these cotton event. Also included are DNA sequences which comprise 15 or more nucleotides of contiguous insert sequence from cotton event MON15985X and at least one nucleotide of flanking DNA from cotton event MON15985X adjacent to the insert sequence. Such DNA sequences are diagnostic for cotton event MON15985X. Nucleic acid amplification of genomic DNA from

the event produces an amplicon comprising such diagnostic DNA sequences. In addition, detection the binding of oligonucleotides which bind specifically to the diagnostic sequences described herein is also diagnostic for the event.

According to another aspect of the invention, cotton plants and seeds comprising such DNA sequences which are diagnostic for cotton event MON15985X and MON15985 are provided. The cotton seeds comprising sequences diagnostic for the presence of cotton event MON15985X and MON15985 DNA were deposited on September 29, 2000 with the American Type Culture Collection and are designated as ATCC accession number PTA-2516.

According to another aspect of the invention, oligonucleotide sequence primer pairs for distinguishing cotton event MON15985 and MON15985X from the native, nontransformed, and undisturbed sequence within the wild type sequence within the genome of at least a variety of cotton comprising DeltaPine 50 are provided. In particular, such flanking sequence primers pairs comprise at least one isolated nucleic acid comprising at least 15 contiguous nucleotides from about base pair positions 1-361 as set forth in SEQ ID NO:11, the sequence disclosed herein as the cotton genomic DNA sequence flanking the 5' end of the inserted DNA as depicted in Figure 1, and at least one isolated nucleic acid comprising at least 15 contiguous nucleotides from about base pair positions 674-1361 as set forth in SEQ ID NO:11, the sequence disclosed herein as the cotton genomic DNA sequence flanking the 3' end of the inserted DNA as depicted in Figure 1. In addition, flanking sequence primer pairs for detecting cotton event MON15985 and event MON15985X DNAs, and for distinguishing DNA from these events from cotton DNA not containing sequences diagnostic for these events are provided. Such flanking sequence primer pairs comprise at least one isolated nucleic acid comprising at least 15 contiguous nucleotides from nucleotides 1-1885 of SEQ ID NO:11 paired with at least one isolated nucleic acid comprising at least 15 contiguous nucleotides from nucleotides 362-2267 of SEQ ID NO:11, or primer pairs comprising at least one isolated nucleic acid comprising at least 15 contiguous nucleotides from nucleotides 1-673 of SEQ ID NO:12 paired with at least one isolated nucleic acid comprising at least 15 contiguous nucleotides from nucleotides 350-1361 of SEQ ID NO:12.

According to another aspect of the invention, primer sets that are useful for nucleic acid amplification, for example, are provided. Such primer sets comprise the above-described

flanking sequence primers and an isolated nucleic acid comprising at least 15 contiguous nucleotides of insert sequence of the cotton events MON15985 and MON15985X. For example, SEQ ID NO:26 and SEQ ID NO:27 produce an amplicon which is diagnostic for the presence of cotton event MON15985X DNA, and SEQ ID NO:28 and SEQ ID NO:29 produce yet another amplicon which is diagnostic for the presence of cotton event MON15985X DNA. In contrast, SEQ ID NO:26 and SEQ ID NO:29 are capable of producing an amplicon from DNA samples obtained from other than cotton event MON15985X or MON15985, and from hemizygous genomes comprising the wild type sequence at the point at which the MON15985 sequence integrated into the genome as well as the MON15985 integrant sequence, and said amplicon would appear to be diagnostic for the lack of cotton event MON15985X or MON15985 DNA in a sample, and produces no amplicon in a sample containing . Caution should be exercised in interpreting such a result, however, and one skilled in the art would recognize that when present as a hemizygous member of a chromosome pair, the use of SEQ ID NO:26 and SEQ ID NO:29 as a diagnostic tool for proving a negative or a positive may be erroneous.

According to another aspect of the invention, methods of detecting the presence of DNA corresponding to the cotton event MON15985 or MON15985X in a sample are provided. Such methods comprise: (a) contacting the sample comprising DNA with a primer set that, when used in a nucleic-acid amplification reaction with genomic DNA from cotton event MON15985 or MON15985X, produces an amplicon that is diagnostic for cotton event MON15985X; (b) performing a nucleic acid amplification reaction, thereby producing the amplicon; and (c) detecting the amplicon.

According to another aspect of the invention, methods of detecting the presence of DNA corresponding to the cotton event MON15985X in a sample, such methods comprising: (a) contacting the sample comprising DNA with a probe that hybridizes under stringent hybridization conditions with genomic DNA from cotton event MON15985X and does not hybridize under the stringent hybridization conditions with DNA from a control cotton plant; (b) subjecting the sample and probe to stringent hybridization conditions; and (c) detecting hybridization of the probe to the DNA.

Another aspect of the present invention is methods and compositions for detecting the presence of a target site, i.e., at least one of the junctions, identification or detection of which

would be diagnostic for the presence of the integrated DNA with the genomic of the cotton plant in the cotton event MON15985X, in a sample of nucleic acid derived from or obtained from the genome of the cotton event MON15985X, using a variety of detection methods including TAQMAN (Perkin Elmer) or related fluorophore/quencher methodologies, thermal amplification, ligase chain reaction, southern hybridization, ELISA methods, and colorimetric and fluorescent detection methods. In particular, the present invention provides for kits for detecting the presence of the target sequence, i.e., at least one of the junctions of the insert DNA with the genomic DNA of the cotton plant in the cotton event MON15985X, in a sample containing genomic nucleic acid from the cotton event MON15985X. The kit is comprised of at least one polynucleotide capable of binding to the target site or substantially adjacent to the target site and at least one means for detecting the binding of the polynucleotide to the target site. The detecting means can be selected from the group consisting of fluorescent, chemiluminescent, colorimetric, or isotopic and can be coupled at least with immunological methods for detecting the binding. A kit is also envisioned which can detect the presence of a target site in a sample, i.e., at least one of the junctions of the insert DNA with the genomic DNA of the cotton plant in the MON15985 event, taking advantage of two or more polynucleotide sequences which together are capable of binding to nucleotide sequences adjacent to or within about 100 base pairs, or within about 200 base pairs, or within about 500 base pairs or within about 1000 base pairs of the target sequence and which can be extended toward each other to form an amplicon which contains at least the target site.

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

DRAWINGS

Figure 1 illustrates a summary of the sequence alignment for the DNA flanking the inserted sequence encoding Cry2Ab in cotton event MON15985. The inserted DNA is depicted as described herein, 5' from the left through 3' to the right. Individual DNA sequences identified herein are labeled accordingly. 1) corresponds to the position of the 5' end flanking genome sequence as set forth in SEQ ID NO:19; 2) corresponds to a diagnostic sequence as set forth in SEQ ID NO:14 between the 5' end flanking genome sequence and the

following chloroplast related DNA sequence; 3) corresponds to the position of the chloroplast related sequence positioned 5' the inserted sequence as set forth in SEQ ID NO:20; 4) corresponds to a diagnostic sequence as set forth in SEQ ID NO:15 between the 3' end of the chloroplast related DNA sequence and the following remnant cotton genome related sequence; 5) corresponds to the position and sequence of the 5' end remnant of cotton genome sequence positioned immediately 3' to the 5' end of the inserted sequence; 6) corresponds to a diagnostic sequence as set forth in SEQ ID NO:16 between the 3' end of the 5' end remnant cotton genome sequence and the 5' end of the inserted DNA sequence; 7) corresponds to the position of a portion of the 5' end of the inserted sequence as set forth in SEQ ID NO:22; 8) corresponds to the position of a portion of the 3' end of the inserted sequence as set forth in SEQ ID NO:23; 9) corresponds to a diagnostic sequence as set forth in SEQ ID NO:17 between the 3' end of the inserted sequence and the 5' end of a 3' end related remnant cotton genome sequence; 10) corresponds to the position of the 3' end related remnant cotton genome sequence; 11) corresponds to a diagnostic sequence as set forth in SEQ ID NO:18 between the 3' end of the 3' end related remnant cotton genome sequence and the 3' genome sequence flanking the inserted DNA sequence; 12) corresponds to the position of the sequenced portion of the 3' end cotton genome flanking the 3' end of the inserted DNA sequence, as set forth in SEQ ID NO:25.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Cotton, *Gossypium hirsutum*, has been genetically modified to resist *Lepidopteran* pests, which have a negative impact on cotton production. This was accomplished by the insertion of a first DNA cassette that encodes the insecticidal Cry1Ac protein from *Bacillus thuringiensis* and a second DNA cassette that encodes the insecticidal Cry2Ab protein from *Bacillus thuringiensis*. This invention relates preferably to plants, plant parts, progeny plants which contain at least the sequences comprising the second DNA cassette, and to methods and compositions of matter for use in detecting the presence of said sequences in a sample.

The first DNA cassette was inserted into the genome of cotton cultivar Coker 312 through *Agrobacterium sp* transformation using a DNA fragment derived from plasmid PV-GHBK04 (pMON10518 in U.S. Patent No. 5,500,365) to produce cotton event MON531, which has been down bred with many different cotton varieties and successfully introduced in

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cotton markets around the world. Cotton event MON531 was retransformed using particle acceleration technology with a gel purified linear DNA fragment, i.e., the second cassette indicated herein and above, from plasmid PV-GHBK11, which contained cry2Ab and β -glucuronidase (uidA) coding regions. (John, M.E. 1997. *Cotton Crop Improvement Through Genetic Engineering*. Critical Reviews in Biotechnology, 17 (3): 185-208). The uidA was used as a selectable marker to aid in identifying cells which contained the cry2Ab2 coding region. The cry2Ab coding region derived from plasmid PV-GHBK11 comprises the cauliflower mosaic virus (CaMV) promoter with a duplicated enhancer region (U.S. Patents 5,530,196; 5,424,200; and 5,359,142) operably connected to a petunia heat shock protein untranslated leader sequence (PetHSP70-leader) operably connected or linked to the N-terminal chloroplast transit peptide from *Arabidopsis thaliana* EPSPS gene (AEPSPS/CTP2) (Van den Broeck, et. al. 1985. *Targeting of a foreign protein to chloroplasts by fusion to the transit peptide from the small subunit of ribulose 1,5-bisphosphate carboxylase*. Nature 313, 358-63.) operably connected or linked to a synthetic sequence encoding Cry2Ab (Widner, W.R. and Whiteley, H.R. 1990. *Location of the Dipteran Specificity Region in a Lepidopteran-Dipteran Crystal Protein from Bacillus thuringiensis*. J Bacteriol. 172, 2826-32.) which is operably connected or linked to the 3' nontranslated region of the nopaline synthase (NOS) gene from *Agrobacterium tumefaciens* which terminates transcription and directs polyadenylation (Fraley, R.T., et al. 1983. *Expression of bacterial genes in plant cells*. Proc Natl Acad Sci U.S.A., 80 (15), 4803-07.). The β -glucuronidase coding region is also controlled by an enhanced CaMV 35S promoter and a NOS 3' polyadenylation sequence. The insertion of the cassette, or substantially all of the cassette, containing the Cry2Ab and UidA coding sequences into the cotton event MON531 gave rise to a single event designated as MON15985 which comprises both the Cry1A coding sequence as well as the cassette encoding the Cry2Ab coding sequence. Because the cassettes encoding these proteins were introduced in separate transformations, and because the cotton genome consists of several chromosomes comprising the total nuclear genome, the likelihood that the second cassette would insert near to or within the same chromosome as the Cry1A coding sequence was very low. Furthermore, genetic analysis of the MON15985 event has demonstrated that the two inserted cassettes can be segregated, the segregants giving rise to the original MON531 event genotype as well as a second genotype designated as MON15985X comprising only the Coker

312 genetic background containing a single cassette incorporated into the nuclear genome which is responsible for encoding the Cry2Ab protein. It is the transformation event giving rise to the cotton genotype MON15985 as well as the MON15985X genotype which are intended as the subject matter of this invention.

- 5 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*
 10 *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."

15 Event

- As used herein a transgenic "event" refers to a recombinant plant produced by transformation of plant cells with heterologous DNA, i.e., a nucleic acid construct that includes a transgene of interest, 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
 20 characterized by insertion into a particular genome location. The term "event" refers to the original transformant and progeny of the transformant that include the heterologous DNA. 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 backcrossing to a recurrent parent, the inserted DNA and flanking DNA from the transformed
 25 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
 30 transformant and progeny resulting from selfing) and a parental line that does not contain the inserted DNA.

Probes and Primers

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 genomic DNA from cotton event MON15985 (whether from a cotton 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 polyanides 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 or sets can be used for amplification of a nucleic acid sequence, e.g., by the polymerase chain reaction (PCRTM), also known as thermal amplification methods, or other conventional nucleic-acid amplification methods.

Probes and primers are generally 15 nucleotides or more in length, preferably 20 nucleotides or more, more preferably 25 nucleotides, and most preferably 30 nucleotides or more. 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 sequence complementarity with the target sequence, although probes differing from the target sequence and which retain the ability to hybridize to target sequences may be designed by conventional methods.

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, NY, 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. PCRTM-primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © 1991, Whitehead Institute for Biomedical Research, Cambridge, MA).

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.

Nucleic-Acid Hybridization: "Stringent Conditions"; "Specific"

- 5 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.

- The term "stringent conditions" is functionally defined with regard to the
10 hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic-acid sequence of interest) by the specific hybridization procedure discussed in Sambrook *et al.*, 1989, at 9.52-9.55. *See also*, Sambrook *et al.*, 1989 at 9.47-9.52, 9.56-9.58; Kanahisa, *Nucl. Acids Res.* 12:203-213, 1984; and Wetmur and Davidson, *J. Mol. Biol.* 31:349-370, 1968.

- Regarding the amplification of a target nucleic-acid sequence (*e.g.*, by PCRTM) using
15 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 term "specific for (a target sequence)" indicates that a probe or primer hybridizes
20 under stringent hybridization conditions only to the target sequence in a sample comprising the target sequence.

Nucleic-Acid Amplification

- 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
25 example, to determine whether the cotton plant resulting from a sexual cross contains an transgenic event genomic DNA from a cotton plant may be subjected to nucleic acid amplification using a primer pair that includes a primer derived from flanking sequence in the genome of the plant 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
30 for the presence of the event (*e.g.*, the amplicon is of a length and has a sequence that is diagnostic for the event). Alternatively, a primer pair can be derived from flanking sequence

on both sides of the inserted DNA so as to produce an amplicon that includes the entire insert.

Nucleic-acid amplification can be accomplished by any of the various nucleic-acid amplification methods known in the art, including the polymerase chain reaction (PCRTM). A variety of amplification methods are known in the art and are described, *inter alia*, in U.S. Patent 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. Any well known method for nucleic acid amplification may be used in the practice of the present invention.

The sequence of the heterologous DNA insert or flanking sequence from cotton event MON15985 can be verified (and corrected if necessary) by amplifying such sequences from the event using primers derived from the sequences provided herein followed by standard DNA sequencing.

Detection Kit:

As used herein, "detection kit" refers to a kit used to detect the presence or absence of DNA from a MON15985 event in a sample comprising nucleic-acid probes and primers of the present invention, which hybridize under stringent conditions to a target DNA sequence, and other materials necessary to enable nucleic acid hybridization or amplification methods. Alternatively, a detection kit may comprise materials necessary to enable one skilled in the art to perform methods similar to those described in PCT International Application WO 97/22719, incorporated herein by reference, to detect the presence or absence of DNA from a MON15985 event in a sample.

Example 1

Cotton, *Gossypium hirsutum*, has been genetically modified to resist *Lepidopteran* pests, which have a negative impact on cotton production. This was accomplished as indicated herein by the insertion of a DNA cassette which encodes the insecticidal Cry1Ac protein from *Bacillus thuringiensis* into the genome of cotton cultivar Coker 312 through *Agrobacterium sp* transformation using a DNA fragment derived from plasmid PV-GHBK04 (pMON10518 in U.S. Patent No. 5,500,365). This transformation resulted in three separate insertions into the cotton genome. The primary, functional insert responsible for expressing the Cry1Ac protein in cotton event 531 comprises a promotor, *cry1Ac* coding region, and

termination sequence linked to a sequence which encodes an antibiotic selectable marker. A second insert adjacent to and immediately upstream of the first inserted sequence comprises a partial *cryIAc* coding region and termination sequence. The third insert comprises only a partial termination sequence and has been shown to segregate independently from the first two sequences indicated above. The first and second inserted sequences are tightly linked and therefore do not segregate from each other. Cotton genome sequence flanks the 5' and 3' ends of all three inserts. Therefore, six unique cotton genome/insert junctions were created as a result of the transformation process.

Molecular analyses were performed on cotton event MON531 to define the ends of the transgene DNA insertions and identify the cotton genomic DNA flanking the transgene DNA inserts. Genome walking studies combined with nucleotide sequencing yielded the DNA sequences of four said cotton genome/insert junctions.

The complex arrangement of genetic elements, derived from the transformation plasmid PV-GHBK04 in cotton event MON531 provides novel nucleic acid sequences at the 5' and 3' cotton genome/insert junctions at each of three sites of insertions. These novel nucleic acid sequences are useful for detecting DNA from cotton event MON531 in a sample using various methods well known in the art. The following provides a nonlimiting example of how one might use these novel nucleic acid sequences to detect cotton event MON531 in a sample.

DNA Isolation for PCR™ Analyses.

DNA from cotton event MON531 was extracted from seed tissue. DNA was extracted from both seed and leaf tissues from the control substance (non-transgenic cotton seed and leaf tissue). DNA from seed was isolated by processing the seed to a fine powder using a commercially available blender. Approximately 2 g of the processed seed was transferred to a 50 ml conical tube, and ~16 ml of CTAB extraction buffer [1.5% (w:w) CTAB, 75 mM Tris-HCl pH 8.0, 100 mM EDTA pH 8.0, 1.05 M NaCl, and 0.75% (w:w) PVP (MW 40,000)] was added to the processed seed. The samples were incubated at 65°C for approximately 30 minutes with intermittent mixing and then allowed to cool to room temperature. An equal volume (~16 ml) of room temperature chloroform:isoamyl alcohol (24:1 (v/v)) or chloroform was added to the samples. The suspension was mixed by

inversion, and the two phases separated by centrifugation at $-16,000 \times g$ for 5 minutes. The aqueous (top) layer was removed using a transfer pipet and placed into a clean 50 ml conical tube. Approximately 1/10 volume (~ 1.6 ml) of 10% CTAB buffer [10% (w:w) CTAB and 0.7 M NaCl] was added to the aqueous phase, which was then mixed by inversion. The samples were centrifuged at $-16,000 \times g$ for 5 minutes to separate the phases. The aqueous (upper) phase was removed, mixed with an equal volume (~ 15 ml) of CTAB precipitation buffer [1% (w:w) CTAB, 50 mM Tris pH 8.0, and 10 mM EDTA pH 8.0] and allowed to stand at room temperature for approximately 1 hour. The samples were centrifuged at $-10,000 \times g$ to pellet the DNA, the supernatant was decanted, and the pellet was dissolved in approximately 2 ml of high salt TE [10mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, and 1 M NaCl] by incubating at 37°C with gentle swirling for approximately 2 hours. Centrifugation was performed at $-23,000 \times g$ to pellet any remaining impurities. The supernatant was removed, placed into a clean 15 ml tube, and approximately 1/10 volume (~ 150 μl) of 3M NaOAc, pH 5.2, and 2 volumes (~ 4 ml relative to the supernatant) of chilled 100% ethanol were added to precipitate the DNA. The precipitated DNA was spooled into a microfuge tube containing approximately 1 ml of 70% ethanol. The DNA was pelleted in a microfuge at maximum speed (14,000 rpm) for 5 minutes, dried, and re-dissolved in TE, pH 8.0 in a 4°C refrigerator overnight.

The non-transgenic cotton genomic DNA used as a control was isolated from leaf tissue that was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. Approximately 1 g of the ground leaf tissue was transferred to a 13 ml centrifuge tube and 6 ml of extraction buffer [2.5 ml DNA extraction buffer (350 mM sorbitol, 100 mM Tris pH 7.5, 5 mM EDTA, 0.38% (w/v) sodium bisulfite), 2.5 ml nuclei lysis buffer (200 mM Tris pH 7.5, 50 mM EDTA, 2 M NaCl, 2% (w/v) CTAB), and 1 ml Sarkosyl (5% (w/v) solution)] was added. The samples were incubated at 65°C for approximately 30 minutes with intermittent mixing. Four and a half milliliters of chloroform:isoamyl alcohol (24:1 (v/v)) at room temperature was added to the samples. The suspension was mixed for 2 to 3 minutes, and the two phases separated by centrifugation for 15 minutes at $-2,000 \times g$ at 4°C . The aqueous (top) layer was removed using a transfer pipet and placed into a 13 ml centrifuge tube. Five milliliters of 100% isopropanol were added, and the tubes were mixed by inversion to precipitate the DNA. The precipitated DNA was spooled into a microfuge tube

containing 500 μ l of 70% ethanol. The DNA was pelleted in a microfuge at maximum speed (14,000 rpm) for 2 minutes. The DNA was dried and dissolved in TE buffer in a 4°C refrigerator overnight.

5 **PCR™ Verification of Unique Insert-Cotton Genome Junctions in Cotton Event MON531.**

10 The DNA sequences of four cotton genome/insert junctions were identified using the PCR™-based Universal Genome Walker Kit™ as per the manufacturer's protocol followed by nucleotide sequencing of the PCR™ products. Next, PCR™ assays were developed using one primer complementary to cotton genomic DNA and another primer complementary to inserted transgene DNA. For example, one primer designed to the 3' end of the primary, functional insert complementary to genomic flanking sequence (e.g., SEQ ID NO:6) paired with a second primer at the 3' end of the primary, functional insert complementary to inserted transgene sequence (e.g., SEQ ID NO:5). The PCR™ assays were performed using 10 - 100 ng of cotton event MON531 genomic DNA template in a 50 μ l reaction volume containing a final concentration of 1.1 mM Mg^{2+} , 0.4 μ M of each primer, 200 μ M each dNTP, and 2.5 units of Taq DNA polymerase. The reactions for the PCR™ assays were performed under the following cycling conditions: 1 cycle at 94°C for 3 minutes; 38 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C for 90 seconds; 1 cycle at 72°C for 10 minutes. The PCR™ products were separated using agarose gel electrophoresis, visualized by ethidium bromide staining, excised from the gel, and subjected to DNA sequencing using dye-terminator chemistry to confirm the sequences.

20 In the example above, as expected, the control reactions without template DNA and Coker 312 non-transgenic negative control DNA did not generate a PCR™ product. The cotton event MON531 samples generated the expected size PCR™ products of 264 bp for the 3' flanking sequence. Therefore, the novel nucleic acid sequences at the junction of inserted DNA and cotton genomic DNA in cotton event MON531 are useful for detecting DNA derived from cotton event MON531 in a sample.

30 MON531 in the variety Coker 312 background was crossed into the variety DP50 (Delta & Pine Land Company) to produce a Delta&Pine Land Company variety containing the

MON531 event encoding the Cry1A protein. The subsequent variety was designated as DP50B.

EXAMPLE 2

5 This example describes the production of a recombinantly transformed cotton plant designated as MON15985 derived from DP50B which contains an additional insertion event encoding a Cry2Ab insect inhibitory protein, and the isolation and characterization of the 5' and 3' DNA sequences flanking the 5' and 3' ends of the MON15985 and segregant MON15985X insertion event.

10 Target DP50B plant cells were transformed using particle acceleration technology with a linear DNA segment derived from plasmid PV-GHBK11. The linear DNA segment was prepared by digestion of plasmid PV-GHBK11 with the restriction enzyme KpnI, separation of the plasmid fragments by agarose gel electrophoresis and isolation of the DNA segment containing cassette comprising the Cry2Ab coding sequence and UidA coding
15 sequence (GUS). No plasmid backbone was contained within the segment or sequence isolated and purified and prepared for bombardment of cotton tissue. The expression cassette consists of a Cry2Ab coding region regulated by an enhanced CaMV35 S plant expressible promoter and an Agrobacterium tumefaciens NOS3' transcription termination and polyadenylation sequence. The Cry2Ab coding sequence is designed to target the expression
20 product as a precursor protein for chloroplast import in plant cells (US Patent Application Serial NO. 09/186,002, filed November 4, 1998).

The cotton variety DP50B was transformed with the DNA cassette described above containing a sequence encoding a Cry2Ab insect inhibitory protein by bombardment with DNA coated gold particles using electrostatic discharge method (John, M.E., Cotton Crop
25 Improvement Through Genetic Engineering. Critical Reviews in Biotechnology, 17:185-208 (1997)). Different events were screened for efficacy against insect pests, phenotype, and genetic segregation for expression of the Cry2Ab protein. Based on these criteria, 6 lines were chosen for further characterization of the insertion event. Southern blot was used as the primary method for evaluating the presence of one or more copy numbers of insertion and
30 completeness of the insertion event. One line, 15985, was chosen for a thorough molecular

characterization of the insertion event that inserted into the DP50B genome. The DNA surrounding the insertion event was isolated and characterized.

Isolation of the DNA flanking the insertion event: DNA was extracted from young cotton leaves by a modified Phyto-Pure® method from Amersham which is designed to extract DNA from plants with large amounts of contaminating carbohydrates. In order to determine the sequence of the DNA around the inserted Cry2Ab coding sequence, the Genome Walker® protocol from Clontech, Inc. was used. The manufacturer's recommended conditions were followed with only one modification: rather than using phenol extraction for purification of the DNA after restriction endonuclease cleavage, the DNA was purified with a Qiagen QIAquick® spin column in accordance with the manufacturer's instruction manual.

The enzymes used to cut the genomic DNA prior to linker ligation were *DraI*, *PvuII*, *ScaI* and *SstI*. Following the procedure provided by Qiagen, the DNA was extracted in 5 volumes of PB buffer, bound to the column, washed with 0.75ml of PE and then eluted from the column into 20mL of 10mM Tris pH 8.5. This DNA was then used as the source for the linker libraries. Primer positions were deliberately chosen away from the ends of where the DNA was linearized to allow for a certain amount of deletion at either end. The first primers (SEQ ID NO:30 [gactgaatgc ccacagccg tcgagt] and SEQ ID NO:31 [cctgaactt gccaccagt tcgacc]) were designed to anneal to sequences that are unique to a Cry2Ab coding sequence because of the presence of repeated DNA elements in the cotton genome. The resulting PCR™ products were subjected to a second PCR™ reaction using nested primers directly adjacent to those used in the first reaction (SEQ ID NO:32 [ggtttctaca ggacggacca tggagaatgc] and SEQ ID NO:33 [ccaactaaca tctcgccgct gtactgatag g]). The PCR™ parameters used were those recommended by Clontech. The primary amplification parameters were as follows: 7 cycles 94°C 2 sec, 72°C 3min, 32 cycles 94°C 2 sec, 68°C 3 min, and secondary amplification parameters were as follows: 5 cycles 94°C 2 sec, 72°C 3 min, 20 cycles 94°C 2sec, 68°C 3 min, and 68°C 4 min in last cycle. All PCR™ reactions were performed on a Perkin-Elmer 9700 machine.

The resulting PCR™ products from the second amplification were cloned into the pBS(SK+) vector from Stratagene which had been cut with *SmaI* to generate a blunt end and treated with alkaline phosphatase to prevent self-ligation. The DNA fragments that were

generated in the secondary PCRTM reaction were treated with T4 DNA kinase to add a 5'phosphate and then ligated into the blunt end vector under standard ligation conditions.

Genome Walker[®] protocol: The secondary PCRTM reactions using the nested primer pairs produced multiple fragments synthesized from both 5' and 3' ends. Three independent fragments for the 5' end were cloned and sequenced using methods well-known in the art, while two fragments were cloned and sequenced from the 3' end. For each individual fragment, at least two independent clones were obtained.

The sequence of the clones from each primer set were compared to one another. The sequences among the respective clones were determined to be the same, and contained the same plant trans-gene junction.

EXAMPLE 3

This example illustrates how the DNA sequences flanking the MON15985 and MON15985X insertion event can be used to determine zygosity.

Design and development of a PCRTM based test for homozygosity of the MON15985 insertion event: The DNA sequence of the two regions flanking the MON15985 insertion event was used to design a PCRTM based homozygosity test. Such a test would be considered useful if it were able to detect both the specific MON15985 insertion event and the wildtype chromosome. To test the feasibility of developing an assay that also detects the wild type chromosome, primers were designed from the sequence directly adjacent to the insertion event to test if they could be used to synthesize a DNA fragment in the non-transgenic cotton, as well as in the 15895 line. The primers chosen were capable of synthesizing the whole MON15985 insertion event, but no product was observed in the non-transgenic controls. These results indicated that the insertion event was not a simple break in the DNA and insertion of the DNA sequence and cassette encoding Cry2Ab. This result, however, established that the two ends are indeed connected and flank an intact gene cassette encoding Cry2Ab.

Primers were designed to the nucleic acid sequence flanking the 3' end of the insert to amplify fragments via the Genome Walker[®] protocol in linker libraries made from non-transgenic cotton DNA. A 620 base pair fragment was cloned and sequenced from non-transgenic cotton. This sequence was then used to design primers for the wild type

chromosome that would be present in the heterozygous individual. Several sets of primers were made and tested together in order to find a set that worked in a single reaction and provided no background bands. A primer set comprising SEQ ID NO:34 ([tggtgcttc tgggctac]3' cotton plant flanking sequence) and SEQ ID NO:35 ([cttgcatg gtgcagacag gtc]5' cotton plant flanking sequence) was found to produce an 800 base pair amplicon from wild type, or non-transgenic cotton DNA. However, if at least one insert is present, the primer comprising SEQ ID NO:36 will anneal to cry2Ab specific sequences and generate a 1.5 kb amplicon with a primer annealing to the 3' cotton plant flanking sequence (SEQ ID NO:34). Therefore, the resulting assay using PCRTM primers comprising SEQ ID NO:36, SEQ ID NO:34, and SEQ ID NO:35 is as follows: if an individual is homozygous for the MON15985 insertion event then only one band of 1.5 kb would be present; but if it is heterozygous then an additional 0.8 kb band would also be present.

Both of these bands (1.5 kb and 0.8 kb) were cloned and sequenced to confirm their identity. The DNA sequence of the 800 base pair fragment generated in heterozygous individuals is identical to the sequence of the Genome Walker[®] fragment generated with the primers from the 3' end and non-transgenic DNA.

Confirmation of PCRTM based zygosity assay: To verify the precision of the zygosity assay a series of experiments were conducted utilizing known homozygous, heterozygous and negative transgenic progeny of MON15985.

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R2:

Phenotype: Fifteen individual R2 plants from three suspected R1 genotypes were characterized for the presence or absence of the Cry2Ab protein via ELISA. Plants of interest that were positive for the study included MON15985-2 and MON15985-34. Plant MON15985-71 was identified as negative for Cry2Ab.

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R3:

Phenotype: Fifteen individual R3 seeds from each of the selected R2 plants were planted in 4" pots and subsequently screened for the presence of the Cry2Ab protein via qualitative ELISA. The ELISA results reported that all R3 plants of MON15985-2 were positive, thus giving the R2 parent a designation of being homozygous for the cry2Ab gene. R3 progeny of MON15985-34 were a mixture of positive and negative

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plants giving the R2 parent a designation of being heterozygous for the *cry2Ab* gene. All R3 plants of MON15985-71 were negative and confirmed that the R2 parent was negative as well.

5 Genotype: A PCRTM-based zygosity assay was performed on five R3 plants of MON15985-2 and MON15985-71. Primers and techniques were used as previously described. Results of this test gave the expected banding patterns. All progeny of MON15985-2 produced only the single 1.5 kb band supporting the ELISA data, which indicated the MON15985-2 line is homozygous positive for the *cry2Ab* gene. All
10 progeny of MON15985-71 produced only the single 0.8 kb band found in the wild-type, thus supporting the ELISA results in demonstrating that this line is homozygous negative for the *cry2Ab* gene. Five progeny of each of fourteen individual R2 families of MON15985-34 were screened as well giving combinations of homozygous, heterozygous and negative plants.

15 R4:

Five individual plants from all three R3 populations were allowed to self pollinate and were taken to harvest. Fifteen R4 progeny from each of the R3 plants tested via PCRTM were planted in 4" pots to confirm the results on the R3 plants. Thus, if the R3 PCRTM result was homozygous then the all the R4 progeny should be
20 homozygous, as well if the R3 PCRTM result was heterozygous the R4 progeny should be a mixture of homozygous, heterozygous and negative plants. All R4 progeny families provided the expected result based on the R3 PCRTM assay. Therefore, the PCRTM based assay provides a durable method for genotyping plants for zygosity.

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EXAMPLE 4

This example illustrates the DNA sequences flanking the 5' and 3' ends of the insert in cotton event MON15985 and MON1598SX.

DNA from MON15985 was extracted from leaf tissue that was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. Approximately 1 g
30 of the ground leaf tissue was transferred to a 13 ml centrifuge tube and 6 ml of extraction buffer [2.5 ml DNA extraction buffer (350 mM sorbitol, 100 mM Tris pH

7.5, 5 mM EDTA, 0.38% (w/v) sodium bisulfite), 2.5 ml nuclei lysis buffer (200 mM Tris pH 7.5, 50 mM EDTA, 2 M NaCl, 2% (w/v) CTAB), and 1 ml Sarkosyl [5% (w/v) solution]] were added. The samples were incubated at 65°C for approximately 35 minutes. Four and a half milliliters of chloroform:isoamyl alcohol [24:1 (v/v)] at room temperature were added to the samples. The suspension was mixed for 2 to 3 minutes, and the two phases separated by centrifugation for 15 minutes at $\sim 2,000 \times g$ at 4°C. The aqueous (top) layer was removed and placed into a 13 ml centrifuge tube. Five milliliters of 100% isopropanol were added, and the tubes were mixed by inversion to precipitate the DNA. The precipitated DNA was spooled into a microfuge tube containing 500 μ l of 70% ethanol. The DNA was pelleted in a microfuge at maximum speed (14,000 rpm) for 2 minutes. The DNA was dried and dissolved in TE buffer in a 4°C refrigerator overnight.

DNA from the DP50 control was extracted from seed tissue. DNA from seed was isolated by processing the seed to a fine powder using a commercially available blender. Approximately 2 g of the processed seed was transferred to a 50 ml conical tube, and ~ 16 ml of CTAB extraction buffer [1.5% (w/v) CTAB, 75 mM Tris pH 8.0, 100 mM EDTA pH 8.0, 1.05 M NaCl, and 0.75% (w/v) PVP (MW 40,000)] were added to the processed seed. The samples were incubated at 65°C for approximately 30 minutes with intermittent mixing and then allowed to cool to room temperature. An equal volume (~ 15 ml) of room temperature chloroform:isoamyl alcohol [24:1 (v/v)] was added to the samples. The suspension was mixed for 5 minutes, and the two phases separated by centrifugation at $\sim 16,000 \times g$ for 5 minutes at room temperature. The aqueous (top) layer was removed and placed into a clean 50 ml conical tube. Approximately 1/10 volume (~ 1.5 ml) of 10% CTAB buffer [10% (w/v) CTAB and 0.7 M NaCl] and an equal volume of chloroform:isoamyl alcohol [24:1 (v/v)] were added to the aqueous phase, which was then mixed by inversion for 5 minutes. The samples were centrifuged at $\sim 16,000 \times g$ for 5 minutes at room temperature to separate the phases. The aqueous (upper) phase was removed, mixed with an equal volume (~ 15 ml) of CTAB precipitation buffer [1% (w/v) CTAB, 50 mM Tris pH 8.0, and 10 mM EDTA pH 8.0] and allowed to stand at room

temperature for approximately 1 hour. The samples were centrifuged at $-10,000 \times g$ to pellet the DNA, the supernatant was decanted, and the pellet was dissolved in approximately 2 ml of high salt TE [10mM Tris-HCl pH 8.0, 10 mM EDTA pH 8.0, and 1 M NaCl] by incubating at 37°C with gentle swirling for approximately 2 hours or by sitting in a 4°C refrigerator overnight. Centrifugation was performed at $\sim 23,000 \times g$ to pellet any remaining impurities. The supernatant was removed, placed into a clean 15 ml tube, and approximately 1/10 volume ($\sim 150 \mu\text{l}$) of 3M NaOAc, pH 5.2, and 2 volumes ($\sim 4 \text{ ml}$ relative to the supernatant) of 100% ethanol were added to precipitate the DNA. The precipitated DNA was spooled into a microfuge tube containing approximately 1 ml of 70% ethanol. The DNA was pelleted in a microfuge at maximum speed (14,000 rpm) for 5 minutes, dried, and re-dissolved in TE, pH 8.0 in a 4°C refrigerator overnight.

The DP50 and MON15985 DNA were quantitated prior to the initiation of the study within the study from which they were derived. DNA quantitation was conducted using a Hoefer DyNA Quant 200 Fluorometer using Boehringer Mannheim molecular size marker IX as a DNA calibration standard.

PCR analysis of the genomic sequence flanking the 5' end of the insert encoding Cry2Ab in cotton event MON15985 was performed using one primer derived from the 5' genomic flanking sequence paired with a second primer located in the inserted DNA near the 5' end in the enhanced CaMV 35S promoter sequence, spanning a 1894 bp region (SEQ ID NO:26 and SEQ ID NO:27). The PCR analysis for the genomic sequence flanking the 3' end of the insert encoding Cry2Ab in cotton event MON15985 was conducted using one primer located in the NOS 3' polyadenylation sequence near the 3' end of the insert paired with a second primer derived from the 3' genomic flanking sequence, spanning a 763 bp region (SEQ ID NO:28 and SEQ ID NO:29).

The PCR analyses were conducted using genomic DNA from the cotton event MON15985 or non-transgenic cotton line DP50. The PCR analysis was conducted using 35-50 ng of cotton event MON15985 or non-transgenic line DP50 genomic DNA template in a 50 μl reaction volume containing a final concentration of 1.5 mM Mg^{2+} , 0.2 μM of each primer, 200 μM each dNTP, and 2.5 units of Platinum *Taq*

DNA polymerase (Gibco BRL). The reactions for the 5' end of the insert were performed under the following cycling conditions: 1 cycle at 94°C for 3 minutes; 35 cycles of 94°C for 1 minute, 56°C for 1 minute, 72°C for 2 minutes; 1 cycle at 72°C for 10 minutes. The reactions for the 3' end of the insert were performed under the following cycling conditions: 1 cycle at 94°C for 3 minutes; 35 cycles of 94°C for 1 minute, 53°C for 1 minute, 72°C for 45 seconds; 1 cycle at 72°C for 10 minutes. The PCR products were separated on a 1.0 % agarose gel. Electrophoresis was conducted for approximately 1.5-2 hours at 100 V and visualized by ethidium bromide staining.

PCR products of the expected sizes containing the sequences flanking the 5' and 3' ends of the insert encoding Cry2Ab in cotton event MON15985 generated with two primer pairs were isolated by gel electrophoresis of 20 µl of the PCR products on 1.0% agarose gels. PCR products representing the 5' or 3' flanking sequences were excised from a gel and purified using the QIAquick Gel Extraction Kit (Qiagen) following the procedure supplied by the manufacturer. For both analyses, the purified PCR products were then sequenced with the PCR primers using dye-terminator chemistry by the Monsanto Company Genomics Sequencing Center. Due to the length of the PCR products, sequencing was performed with both the primers used to generate the products as well as primers designed internal to the amplified sequence.

PCR analyses were performed on genomic DNA extracted from cotton event MON15985 and non-transgenic cotton line DP50 to verify the DNA sequences flanking the 5' and 3' ends of the insert in cotton event MON15985. The control reaction containing no template DNA as well as the reaction containing DP50 non-transgenic cotton DNA did not generate a PCR product, as expected. PCR analysis of the cotton event MON15985 DNA generated the expected size product of 1894 bp representing the 5' flanking sequence and a portion of the 5' end of the insert encoding Cry2Ab.

The control reaction containing no template DNA as well as the reaction containing DP50 non-transgenic cotton DNA did not generate a PCR product, as expected. PCR analysis of the cotton event MON15985 DNA generated the expected size product of 763 bp representing the 3' flanking sequence and a portion of the 3' end of the insert. These results demonstrate that a predicted size PCR product is

generated from both ends of the insert encoding Cry2Ab in the cotton event MON15985.

The consensus sequence representing the cotton genomic DNA sequence flanking the 5' end of the insert, as well as the DNA at the 5' end of the insert, is represented by SEQ ID NO:11. The 5' consensus sequence data contains 1877 bp of DNA flanking the insert followed by 390 bp of insert sequence containing the enhanced CaMV 35S promoter. Base pairs 362-750 show homology to chloroplast DNA. The consensus sequence representing the cotton genomic sequence flanking the 3' end of the insert, as well as the DNA at the 3' end of the insert, is represented in SEQ ID NO:12. The 3' consensus sequence data contains 349 bp of insert sequence containing the 3' end of the NOS 3' polyadenylation sequence and polylinker sequence followed by 1012 bp of cotton genomic DNA flanking the insert. The reported 5' and 3' consensus sequences presented here are the combination of multiple sequencing reactions and are shorter than those of the PCR products used to generate the sequences. These data delineate the 5' and 3' ends of the insert in the cotton event MON15985 and show the DNA which immediately flanks the insert on both ends.

This data demonstrates that cotton event MON15985 contains a single DNA insert containing (1) a sequence encoding UidA (GUS) regulated by the enhanced 35S CaMV promoter (lacking approximately 260 bp at the 5' end) and NOS 3' polyadenylation sequence; and (2) a coding sequence encoding Cry2Ab regulated by the enhanced 35S CaMV promoter and the NOS 3' polyadenylation sequence (Figure 1). PCR and sequence analyses performed in this example confirm the sequence of the 5' and 3' ends of the insert encoding Cry2Ab in the cotton event MON15985, and verify the genomic DNA sequence flanking the 5' and 3' ends of the insert.

EXAMPLE 5

This example illustrates the physical characteristics of the sequences flanking the cotton event MON15985, and characterizes the nature and physical location of additional DNA sequences flanking the 5' and 3' end of the inserted DNA in the cotton event MON15985. Genomic DNA was analyzed by Southern blotting to determine

the number of insertion events, the copy number of the inserted DNA, the integrity of the inserted promoters, coding regions, and polyadenylation sequences, and the presence or absence of plasmid backbone sequence. All analyses were performed with both the cotton line DP50B (control) and with the newly produced MON15985 event to characterize the newly inserted DNA. In addition, the flanking sequence of the 5' and 3' "insert-to-plant" junctions (previously determined by Genome Walking) were confirmed by PCR.

Plasmid PV-GHBK11, the source plasmid, served as the primary reference substance in these analyses. The plasmid, mixed with DNA from the DP50 control substance, was used as a size indicator and a positive hybridization control in Southern blot analysis. Additionally, molecular size markers from Boehringer Mannheim [Molecular Weight Markers II (23.1 Kb-0.6 Kb) and IX (1.4 Kb-0.072 Kb), catalog #236 250 and #1449 460, respectively] and Gibco BRL [High Molecular Weight DNA Marker (48.5 Kb-8.3 Kb) and 100 bp ladder (2.1 Kb-0.1Kb), catalog #15618-010 and #15628-019, respectively] were used for size estimations.

Genomic DNA from insect protected cotton event MON15985 was digested with a variety of restriction enzymes and subjected to Southern blot hybridization analysis to characterize the DNA encoding Cry2Ab2 and GUS integrated into the genome of DP50B.

DNA extracted from leaf tissue was used for all of the analyses in this example except for the nontransgenic sample on the *uidA* gene cassette intactness blot probed with the NOS 3' polyadenylation sequence probe which was isolated according to the method of Rogers and Bendich (1985). Leaf tissue was frozen in liquid nitrogen and ground into a fine powder using a mortar and pestle. Approximately 1 g of the ground leaf tissue was transferred to 13 ml centrifuge tube containing 6 ml of the extraction buffer [2.5 ml DNA extraction buffer (350 mM Sorbitol, 100 mM Tris pH 7.5, 5 mM EDTA), 2.5 ml Nuclei lysis buffer (200 mM Tris pH 7.5, 50 mM EDTA, 2 M NaCl, 2% CTAB), and 1 ml Sarkosyl (5% solution)]. The samples were incubated at 65°C for approximately 30 minutes with intermittent mixing. Four and a half milliliters of a mixture of chloroform:isoamyl alcohol (24:1) at room temperature was added to the samples. The suspension was mixed for 2 to 3 minutes, and the two phases separated

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by centrifugation for 15 minutes at $\sim 1,000 \times g$ at 4°C . The aqueous (top) layer was removed using a transfer pipet and placed into a 13 ml centrifuge tube. Five milliliters of 100% isopropanol were added, and the tubes were mixed by inversion to precipitate the DNA. The precipitated DNA was pelleted by centrifuging at $\sim 1,000 \times g$ for 5 minutes at 4°C . The pellet was washed with approximately 1 ml of 70% ethanol and centrifuged for an additional 5 minutes at $\sim 1,000 \times g$ at 4°C . The DNA was allowed to dry at room temperature and re-dissolved in TE at 4°C overnight.

The purified genomic DNA was quantitated using a Hoefer DyNA QuantTM 200 Fluorometer (San Francisco, CA) with Boehringer Mannheim Molecular Weight Marker IX used as a calibration standard.

Approximately 10 μg of genomic DNA from the test and control lines were used for the restriction enzyme digests. Overnight digests were performed at 37°C according to manufacturers protocols in a total volume of 500 μl using 100 units of restriction enzyme. Some of the control digests were spiked with either 5 or 10 μg of PV-GHBK11. All restriction enzymes were purchased from Boehringer Mannheim. After digestion, the samples were precipitated by adding 1/10 volume ($\sim 50 \mu\text{l}$) of 3M NaOAc and 2 volumes ($\sim 1 \text{ ml}$ relative to the original digest volume) of 100% ethanol, followed by incubation at -20°C for at least one hour. The digested DNA was pelleted by centrifugation, washed with 70% ethanol, vacuum dried for 10-20 minutes, and re-dissolved at room temperature in either water or TE.

Digested DNA's were separated on 0.8% agarose gels in 1X TBE buffer. A 'long run' and a 'short run' were performed for each Southern blot analysis. The long run facilitated greater resolution of the higher molecular weight DNAs while the short run ensured that all smaller molecular weight DNAs were retained on the gel. The long run/short run involved a 4-6 hour electrophoresis at 80-85 V and an overnight (9-15 hour) run at 35-38 V. After electrophoresis, the gels were stained in 0.5 $\mu\text{g/ml}$ ethidium bromide for 20-30 minutes and photographed.

Plasmid PV-GHBK11 DNA was isolated from overnight *E. coli* cultures. Probe templates homologous to the *cry2Ab* coding region, *uidA* coding region, the enhanced CaMV 35S promoter, the NOS 3' polyadenylation sequence, and the entire backbone region were prepared by PCR using PV-GHBK11 as the template.

Approximately 25 ng of each probe template, except the NOS 3' polyadenylation sequence, were labeled with ^{32}P -dCTP using the random priming method (RadPrime DNA Labeling System, Life Technologies). The NOS 3' polyadenylation sequence was labeled using PCR with NOS 3' template (15 ng), NOS 3' specific primers (0.25 μM each), 1.5 mM MgCl_2 , 3 μM dATP, dGTP, and dTTP, 100 μCi of ^{32}P -dCTP and 2.5 Units of *Taq* DNA polymerase in a final volume of 20 μl . The cycling conditions were as follows: 1 cycle at 94°C for 3 minutes; 5 cycles at 94°C for 45 seconds, 55°C for 30 seconds, and 72°C for 1 minute; 1 cycle at 72°C for 10 minutes. The radiolabeled probe was purified using a Sephadex G-50 column (Boehringer Mannheim).

Southern blot analyses (Southern, 1975) were performed established procedures, generally Maniatis Fritsch Sambrook (Cold Spring Harbor). Following electrophoresis, the gel was incubated in depurination solution (0.125 N HCl) for ~ 10 minutes followed by denaturing solution (0.5 M NaOH, 1.5 M NaCl) for ~30 minutes, and then neutralizing solution (0.5 M Tris-HCl pH 7, 1.5 M NaCl) for ~30 minutes. The DNA from the agarose gels was transferred to Hybond-NTM nylon membranes (Amersham) using a TurboblotterTM (Schleicher & Schuell). The DNA was allowed to transfer for 4 hours to overnight (in 20X SSC) and covalently cross-linked to the membrane with a UV StratalinkerTM 1800 (Stratagene) set to autocrosslink. The blots were prehybridized an average of 2 hours in an aqueous solution of 0.5 M sodium phosphate, 7% SDS (w/v), and 0.1 mg/ml *E. coli* tRNA. Hybridization with the radiolabeled probe was performed in fresh prehybridization solution for 14-21 hours at approximately 65°C. Membranes were washed at least four times in an aqueous solution of 0.1% (w/v) SDS and 0.1X SSC for 15 minute intervals at 65°C. Multiple exposures of the blots were generated using Kodak Biomax MSTM film in conjunction with one Kodak Biomax MSTM intensifying screen. Blots were stripped by incubating the blot with boiling 0.1% (w/v) SDS and allowing it to cool to room temperature.

The insert number (the number of integration sites of newly introduced transgenic DNA in the cotton genome) was evaluated. The test and control DNAs were digested with the restriction enzyme *ScaI*, which does not cleave within the

DNA segment used for transformation. This enzyme released a segment containing the inserted DNA and adjacent plant genomic DNA. The plasmid-spiked DP50 'short run' samples were also digested with *Xba*I to linearize the plasmid. The blot was probed with the reference plasmid PV-GHBK11.

5 The number of copies of the transformation cassette inserted into each locus was determined by digesting the test genomic DNA with the restriction enzyme *Sph*I, an enzyme that cuts only once in the linear DNA segment used to generate the event. The blot was probed with the reference plasmid PV-GHBK11.

10 The integrity of the Cry2Ab coding region was determined by digestion with a restriction enzyme, *Nco*I, that cleaves at the 5' and 3' ends of the Cry2Ab coding region. The blot was probed with the full length Cry2Ab coding region.

15 The integrity of the Cry2Ab encoding cassette (enhanced CaMV 35S promoter, Cry2Ab coding region, and NOS 3' polyadenylation sequence) was assessed by digestion with the restriction enzyme *Bam*HI which cleaves at the 5' and 3' ends of the Cry2Ab cassette. The blot was sequentially probed with each element of the cassette.

20 The integrity of the UidA coding region was determined by digestion with the restriction enzymes *Eco*RI and *Bgl*II which cleave at the 5' and 3' ends of the UidA coding region, respectively. The blot was probed with the full length UidA coding region.

The integrity of the UidA cassette (enhanced CaMV 35S promoter, UidA coding region, and NOS 3' polyadenylation sequence) was assessed by digestion with the restriction enzymes *Bam*HI and *Sph*I which cleave at the 5' and 3' ends of the UidA cassette. The blot was sequentially probed with each element of the cassette.

25 The backbone region of the plasmid is defined as the *Kpn*I restriction fragment of PV-GHBK11 that was not used to transform the plant. It consists of a bacterial origin of replication, ori-pUC, and the *npt*II gene under the control of a bacterial promoter. To confirm the absence of backbone, genomic DNA was digested with the restriction enzyme *Kpn*I and probed with the full-length backbone region.

30 The sequence of the 5' and 3' insert-to-plant genomic DNA junctions were determined as described above using Clontech's Universal Genome Walker™ Kit.

Primers were designed to verify these junctions by PCR. The 5' junction was verified using one primer designed to the 5' genomic flanking sequence paired with a second primer in the enhanced CaMV 35S promoter of the *uidA* gene. The 3' junction was verified using a primer designed to the 3' genomic flanking sequence with a second primer located in the *cry2Ab* gene. The PCRs were conducted using 100 ng of leaf genomic DNA (1-2 µl) as a template, 10 pmol of each primer (1 µl each), and PCR Supermix (Gibco BRL cat no. 10572-014) in a 25 µl reaction volume. The amplification of the reactions was performed under the following cycling conditions: 1 cycle 94°C for 3 minutes; 30 cycles 94°C for 30 seconds, 55°C for 1 minute, 72°C for 2 minutes; 1 cycle 72°C for 4 minutes. The PCR products were separated on a 1% agarose gel in 1X TAE and visualized by staining with ethidium bromide.

Test and control DNA samples were digested with *ScaI*. DP50 control DNA spiked with PV-GHBK11 was also digested with *ScaI*. Since *ScaI* does not cleave within the plasmid, a second enzyme, *XbaI*, was added to linearize the plasmid. The plasmid was linearized to facilitate its migration through the gel to serve as an accurate size estimator. The blot was probed with radiolabeled PV-GHBK11 (Figure 1), the source plasmid for the linear DNA segment used in the transformation. The DP50 long run did not produce any detectable background bands. Plasmid PV-GHBK11 mixed with DP50 short run produced the expected size band at approximately 8.7 Kb, the size of the whole plasmid, with no additional bands. The DP50B long and short runs produced two bands at approximately 22 Kb and 15 Kb (very faint). Since these bands are present in both event MON15985 and the DP50B (MON531) control they are considered background bands associated with the *cryIAC* event. The MON15985 long and short each produced one band not present in either the DP50 or the DP50B lanes at approximately 9.3 Kb. This result suggests that cotton event MON15985 contains one segment of integrated DNA located on a 9.3 Kb *ScaI* restriction fragment.

Genomic DNA isolated from MON15985, DP50B, DP50 (non-transgenic control) and DP50 mixed with plasmid PV-GHBK11 DNA was digested with *SpaI*. The blot was probed with PV-GHBK11, the source plasmid for the linear DNA segment used in transformation. The DP50 long run did not produce any detectable

background bands. Plasmid PV-GHBK11 mixed with DP50 in the short run produced the expected size bands at 3.9 and 4.8 Kb; an additional faint band at 8.7 Kb in lane 5 is presumably due to undigested plasmid DNA. The DP50B long and short runs produced three bands at approximately 6.4, 8.3, and 8.6 Kb. Since these bands are present in both event MON15985 and the DP50B control they are considered background bands associated with the *cry1Ac* event. The MON15985 long and short runs each produced two bands not present in the DP50 or the DP50B lanes at approximately 2.3 Kb and 3.5 Kb. Because the enzyme *SphI* cuts only once within the transformation cassette, this result suggests that MON15985 contains one copy of integrated DNA which produces these two restriction fragments.

DNA from the test, controls, and control mixed with plasmid PV-GHBK11 DNA was digested with *NcoI* to release the Cry2Ab coding region and assess its intactness. The blot was probed with the full-length Cry2Ab coding region. As expected, the DP50 non-transgenic control long run and the DP50B control long and short runs showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 in the short run produced the expected ~1.9 Kb band which corresponds to the entire Cry2Ab coding region. Both the MON15985 long and short runs also produced a 1.9 Kb band which corresponds to the expected size of an intact Cry2Ab coding region. This result establishes that event MON15985 contains the intact Cry2Ab coding region, with no additional detectable fragments.

DNA from the test, controls, and control mixed with plasmid PV-GHBK11 DNA was digested with *BamHI* which releases the entire cassette encoding Cry2Ab (i.e. Cry2Ab coding region, the enhanced CaMV 35S promoter, and the NOS 3' polyadenylation sequence).

The blot was probed with the full length Cry2Ab coding region. The DP50 non-transgenic control long run and the DP50B control long and short runs showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 in the short run produced the expected 3.2 Kb band which corresponds to the entire cassette encoding Cry2Ab. Both the MON15985 long and short runs produced a band at approximately 4.0 Kb. This result indicates that the 3' end of the transformation cassette lost the *BamHI* restriction site during integration into the cotton genome. The

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3' sequence of the insert-to-plant junction, previously determined by genome walking, was verified by PCR analysis. Sixty-six base pairs of the 3' end of the transformation cassette were shown to have been deleted, including the *Bam*HI site. The deleted nucleotides do not include any of the NOS 3' polyadenylation sequence associated with the cassette encoding Cry2Ab, but only linker DNA. These results establish that the cassette encoding Cry2Ab is intact. No partial cassettes derived from that encoding Cry2Ab were detected.

The blot used above was stripped and re-probed with the full length enhanced CaMV 35S promoter. The DP50 long run did not produce any detectable background bands. Plasmid PV-GHBK11 mixed with DP50 in the short run produced the expected size bands at 5.5 and 3.2 Kb with no additional bands detectable. The DP50B long and short runs produced five bands at approximately 4.4, 5.3, 7.5, 9.4, and 22 Kb. Since these bands are present in both event MON15985 and the DP50B control they are considered background bands associated with the *cryI*Ac event. The MON15985 long and short runs both produced one band at approximately 4.0 Kb which is not present in either the DP50 or the DP50B lanes. This corresponds to the fragment predicted for the cassette encoding Cry2Ab given the result obtained with the Cry2Ab coding region probe. A second band in the MON15985 lanes resulting from hybridization to the enhanced CaMV 35S promoter associated with the cassette encoding UidA (GUS) is predicted but not apparent in the test lanes. The results of the NOS 3' polyadenylation sequence probe, discussed below, demonstrate that the enhanced CaMV 35S promoter sequence associated with the UidA encoding cassette is present, but the 4.4 Kb band co-migrates with a 4.4 Kb background band and is not apparent. No extraneous promoters were detected.

The blot used above was re-stripped and re-probed with the full length NOS 3' polyadenylation sequence. The DP50 long run did not produce any detectable background bands. Plasmid PV-GHBK11 mixed with DP50 short run produced the expected size bands at 5.5 and 3.2 Kb with no additional bands detectable. The DP50B long and short runs produced one band at approximately 1.2 Kb. Since this band is present in both event MON15985 and the DP50B control it is considered background associated with the *cryI*Ac event. The MON15985 long and short runs

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each produced two bands which are not present in the DP50 or the DP50B lanes at approximately 4.0 and 4.4 Kb. The 4.0 Kb band corresponds to the fragment predicted for the cassette encoding Cry2Ab, given the result from above. The 4.4 Kb band was not apparent on the blot probed with the enhanced CaMV 35S promoter because it co-migrates with the 4.4 Kb background band seen on that blot. This segment is associated with the *uidA* cassette.

These results establish that the Cry2Ab encoding cassette is intact and that there is a 66 bp deletion between the *Bam*HI site and the 3' end of the transformation cassette, which does not include any of the NOS 3' polyadenylation sequence at the 3' end of the cassette encoding Cry2Ab. No partial cassettes derived from that encoding Cry2Ab were detected.

Genomic DNA isolated from MON15985, DP50B, DP50 (non-transgenic control) and DP50 mixed with plasmid PV-GHBK11 DNA was digested with *Eco*RI and *Bgl*II to release the entire *uidA* coding region. The blot was probed with the full-length *uidA* coding region. The DP50 non-transgenic control long run and the DP50B control long and short runs showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 short run produced the expected ~1.9 Kb band which corresponds to the entire *uidA* coding region. Both the long and short runs of event MON15985 DNA also produced a 1.9 Kb band which corresponds to the expected size of an intact *uidA* coding region. This result establishes that event MON15985 contains the intact *uidA* coding region, with no additional fragments detected.

DNA from the test and control substances was digested with *Bam*HI and *Sph*I to release the entire *uidA* cassette (i.e. *uidA* coding region, the enhanced CaMV 35S promoter, and the NOS 3' polyadenylation sequence). The plasmid PV-GHBK11 was digested with *Pst*I and spiked into the DP50 short run samples after digestion (except for the NOS 3' polyadenylation sequence probe blot in which the plasmid was digested with *Bam*HI and *Sph*I). This was done to show the size of an intact full-length *uidA* cassette.

The blot was probed with the full length *uidA* coding region. As expected, the DP50 non-transgenic control long run and the DP50B control long and short runs

showed no detectable hybridization bands. Plasmid PV-GHBK11 mixed with DP50 short run produced the expected 2.8 Kb band which corresponds to the entire *uidA* cassette. Both the MON15985 long and short runs produced an approximately 2.5 Kb band. This result indicates that a portion of the *uidA* cassette was not present. The 5' insert-to-plant junction, previously determined by genome walking, was verified by PCR analysis. It had been demonstrated previously that 284 bp of the 5' portion of the transformation cassette were deleted. These results establish that the *uidA* cassette is missing approximately 260 bp of the 5' promoter sequence and 24 bp of polylinker DNA derived from the multiple cloning site of the plasmid. Odell et al. (1985) showed that such a deletion should not affect accurate transcription initiation. No additional partial *uidA* cassettes were detected with the *uidA* coding region probe.

The blot used above was stripped and re-probed with the full length enhanced CaMV 35S promoter. The DP50 long run did not produce any background bands. Plasmid PV-GHBK11 mixed with DP50 short run produced the expected size bands at 1.5 and 2.8 Kb with no additional bands detected. The DP50B long and short runs produced five bands at approximately 4.3, 4.6, 5.0, 6.6, and 8.5 Kb. Since these bands are present in both event MON15985 and the DP50B control they are considered background bands associated with the *cry/Ac* event. The MON15985 long and short runs each produced two bands at approximately 2.5 and 1.0 Kb not present in the DP50 or the DP50B lanes. The 2.5 Kb band corresponds to the fragment predicted for the *uidA* cassette. The 1.0 Kb band results from the enhanced CaMV 35S promoter associated with the cassette encoding Cry2Ab. No extraneous promoters were detected.

The blot was probed with the full length NOS 3' polyadenylation sequence. The DP50 long run did not produce any detectable background bands. Plasmid PV-GHBK11 mixed with DP50 short run produced the expected size bands at 3.8 and 2.2 Kb with no additional bands detected. The DP50B long and short run produced one band at approximately 1.2 Kb. Since this band is present in both event 15985 and the DP50B control it is considered background associated with the *cry/Ac* event. The MON15985 long and short runs each produced two bands not present in the DP50 or the DP50B lanes at approximately 2.5 and 2.3 Kb. The 2.5 Kb band corresponds to

the fragment predicted for the *uidA* cassette. The 2.3 Kb band results from NOS 3' polyadenylation sequence associated with the cassette encoding Cry2Ab.

These results confirm that the *uidA* cassette is missing approximately 260 bp of the 5' end of the enhanced CaMV 35S promoter but is otherwise intact.

5 Genomic DNA isolated from event MON15985, DP50B, DP50 (non-transgenic control) and DP50 mixed with plasmid PV-GHDK11 DNA was digested with *KpnI*. The blot was probed with the entire backbone sequence. The DP50 long run showed no detectable hybridization bands. Plasmid PV-GHDK11 mixed with DP50 DNA produced one band at the expected size of 2.6 Kb for the entire backbone. The 10 DP50B long and short run produced a single band at approximately 22 Kb. Since this band is present in both event MON15985 and the DP50B control it is considered background associated with the *cryIAC* event. The MON15985 long and short runs contained the 22 Kb background band with no additional hybridization. This result establishes that event MON15985 does not contain any detectable plasmid backbone 15 sequence.

PCR was performed on genomic DNA to confirm the insert-to-plant junction sequences at the 5' and 3' ends of the MON15985 insert. As expected, the non-transgenic samples did not yield a PCR product when either the 5' or 3' primer set was used. The DP50B sample (*cryIAC* control event) did not yield products with 20 either primer pair, as expected. A different event selected for expression of Cry2Ab, MON15813, also did not yield products when either primer set was used. The MON15985 genomic DNA yielded the correct size products when 5' end using primer pairs and 3' end primer pairs. This PCR analysis confirmed the 5' and 3' border sequences of MON15985.

25 The insect protected cotton event MON15985 was produced by particle acceleration technology using a *KpnI* DNA segment containing a cassette encoding Cry2Ab and *Uida*. The MON15985 event contains a single DNA insert located on a 9.3 Kb *SacI* fragment. This insert contains one complete copy of the inserted cassette which is missing approximately 260 bp at the 5' end of the enhanced CaMV 35S 30 promoter driving expression of the *Uida* coding sequence. PCR was used to verify the 5' and 3' junction sequences of the insert with the plant genome, as well as the

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intactness of the 5' and 3' ends of the insert. Event MON15985 does not contain any detectable plasmid backbone sequence resulting from the transformation event. Based on the enzymes used in this study, the restriction pattern of the *cryI*Ac insert is not changed by the insertion of the DNA inserted comprising the Cry2Ab coding sequence.

Thus, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, and SEQ ID NO:18 and sequences comprising these sequences, are diagnostic for the insertion which gave rise to cotton event MON15985.

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What is claimed is:

1. An insect resistant cotton plant, or parts thereof, seed of said cotton plant having
been deposited with the American Type Culture Collection under accession
number PTA-2516.

5

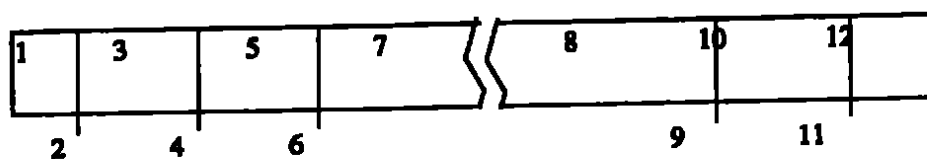
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ABSTRACT

The present invention provides cotton plants, cotton tissues, and cotton seeds that include the MON15985 event, which confers resistance to *Lepidopteran* insect damage. Also provided are assays for detecting the presence of the MON15985 event based on the DNA sequence of the recombinant construct inserted into the cotton genome that resulted in the MON15985 event and of genomic sequences flanking the insertion site.

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Figure 1



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SEQUENCE LISTING

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 sequence
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20

<210> 16
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 <211> 20
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20

<210> 18
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 <223> 3' end cotton genomic remnant sequence adjacent to 3' end Gossypium
 hirsutum chromosome sequence
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<210> 19
 <211> 361
 <212> DNA

<213> *Gossypium hirsutum*
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 tascagcgaa atcttagggt ggtcagagaa atgagattgt taagtctga tttantgtgc 120
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 ttaaaaagc taagytaaa tctgggcasa gttttacgt ggcacagggg aataaaaaag 300
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 <213> unidentified plant
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 chromosome sequence
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 cttaagaaa tagtgaatgy tcttatcaat acacaaatgt ataactccc agaatttca 300
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 <212> DNA
 <213> *Gossypium hirsutum*
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 <213> Gossypium hirsutum
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<210> 25
 <211> 688
 <212> DNA
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 aagagtttc gacccaact agagaagaa ttaaaactgc agaactggc tegtgtgtc 180
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<210> 30
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 <222> (1)..(27)

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<223> completely synthesized
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219

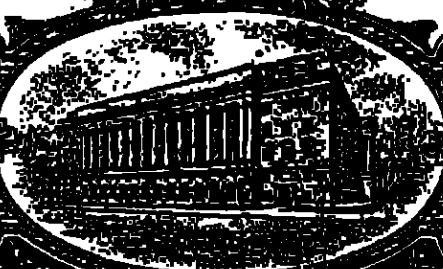
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60297406-061101



LOS ESTADOS UNIDOS DE AMÉRICA

A TODO AQUEL A QUIEN CONCIERNA LO PRESENTE

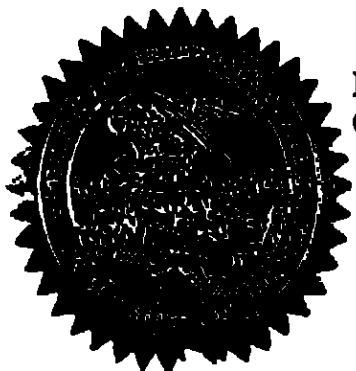
DEPARTAMENTO DE COMERCIO DE LOS ESTADOS UNIDOS

Oficina de Patentes y Marcas de los Estados Unidos

11 de junio de 2002

LA PRESENTE ES PARA CERTIFICAR QUE ADJUNTA A LA MISMA HAY UNA COPIA FIEL DE LOS REGISTROS DE LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS DE LOS DOCUMENTOS DE LA SOLICITUD DE PATENTE IDENTIFICADA EN LO QUE SIGUE, QUE CUMPLEN LOS REQUISITOS PARA OTORGARLE UNA FECHA DE PRESENTACIÓN BAJO 35 USC 111.

NÚMERO DE SOLICITUD: 60/297,406
FECHA DE PRESENTACIÓN: 11 de junio de 2001



**Por la Autoridad del
COMISARIO DE PATENTES Y MARCAS**

P. SWAIN

Oficial certificador

Lo que se reivindica es:

1. Una planta de algodón resistente a insectos, o partes de la misma, semilla de dicha planta de algodón, la cual ha sido depositada con la Colección Americana de Cultivos Tipo bajo el número de acceso PTA-2516.

COLO 12746-801-002-1
MRE

222

Apostille

(Convention de La Haye du 5 octobre 1961)

1. Country: United States of America

- This public document
2. has been signed by MICHELLE LUDWIG
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of MICHELLE LUDWIG-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76728
9. Seal/Stamp
10. Signature:

Matt Blunt

Apostille

(Convention de La Haye du 5 octobre 1961)

1. Country: United States of America

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2. has been signed by NORMA R. POPE
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of NORMA R. POPE-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76726
9. Seal/Stamp
10. Signature:

Matt Blunt

CAVELIER

356

ABOGADOS
EDIFICIO 81810
CARRETERA 48 N° 73-35
BOGOTÁ 8 - COLOMBIA

CESION

ASSIGNMENT

Yo, (nosotros) abajo firmado (s)

I (we), the undersigned

Scott A. Huber, James K. Roberts, Zachary W. Shappley, y Sean Doherty,

Scott A. Huber, James K. Roberts, Zachary W. Shappley, and Sean Doherty,

domicilado(s) en 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South, Collierville, TN 38017 y 1446 Rardin, Richmond Heights, MO 63117, Estados Unidos de América, respectivamente,

of 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South, Collierville, TN 38017 and 1446 Rardin, Richmond Heights, MO 63117, United States of America, respectively,

declaro(amos) bajo juramento ser único(s) y verdadero(s) inventor(es) de la invención:

state(s) under oath to be sole and true inventor(s) of the invention:

PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES Y METODOS PARA DETECCIÓN DEL MISMO

COTTON EVENT MON15985 AND COMPOSITIONS AND METHODS FOR DETECTION THEREOF

declaro(amos) así mismo que cede(amos) y transfiero(amos), sin limitación, a favor de la sociedad MONSANTO TECHNOLOGY LLC

state as well, that assign, without reservations or limitations in favour of MONSANTO TECHNOLOGY LLC

constituida y existente de conformidad con las leyes del estado de Delaware, Estados Unidos de América

a corporation constituted and existing in conformity with the laws of the state of Delaware, United States of America

domiciliada en 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, Estados Unidos de América

domiciled in 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, United States of America

todos los derechos inherentes a dicha invención y que la citada Compañía queda facultada para solicitar en su nombre la patente de invención correspondiente en la República de Colombia.

all rights inherent to said invention and that the above mentioned company hereby is enabled to apply, in its name, for the corresponding patent application, in the Republic of Colombia

Dado y firmado hoy/ Given and signed this _____

en/

X

Firma

Signature

Scott A. Huber

Firma

Signature

Dennis R. Hoerner, Jr.
President & Intellectual Property
Counsel

STATE OF MISSOURI

§

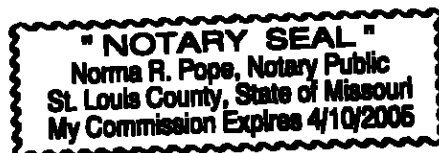
§ ss.

COUNTY OF ST. LOUIS

§

BEFORE ME, the undersigned authority, on this 2nd day of July, 2002, personally appeared Dennis R. Hoerner, Jr., known to me to be the person whose name is subscribed to the foregoing instrument on behalf of Monsanto Technology LLC, and acknowledged to me that he is authorized to validly sign alone such an assignment on behalf of said company.


Notary



AUTENTICACION NOTARIAL (INDIVIDUO)

A los 24 días del mes de Junio de 2002 ante mí, Notario Público de _____ compareció(eron) personalmente Scott A. Huber

a quien(es) doy fe de conocer y me consta que es(son) la(s) persona(s) firmante(s) del documento que precede, y quien(es) tiene(n) capacidad legal para otorgarlo.

Notario Público/Notary Public

NOTARIAL AUTHENTICATION (INDIVIDUAL)

On this 24th day of June 2002 before me, a Notary Public of St. Louis County personally appeared Scott A. Huber

to me known, and known to me to be the person(s) who executed the foregoing document, and who has (have) legal capacity to execute it.

Michelle Ludwig



APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country:
This public document
2. has been signed by
3. acting in the capacity of
4. bears the seal/stamp of

Certified

5. at 6. the
7. by
8. No.
9. Seal/stamp 10. Signature

.....

h
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TRADUCCIÓN OFICIAL No 09.08/02d realizada por Diego Vargas,
Traductor Oficial, Resolución No. 0001 de 1999 del Ministerio de
Justicia y registrado ante el Ministerio de Relaciones Exteriores
de Colombia.

A continuación se traducen los sellos y legalizaciones de un
documento de Cesión escrito en inglés y español por medio del cual
Scott A. Huber, James K. Roberts, Zachary W. Shappley y Sean
Doherty, domiciliados en el 681 Hidden Path, Apt. D, Creve Coeur,
MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597
Waterford Cove South, Collierville, TN 38017 y 1446 Rankin,
Richmond Heights, MO 63117, Estados Unidos de América,
respectivamente, declaran bajo juramento ser únicos y verdaderos
inventores de la invención:

PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES Y MÉTODOS PARA
DETECCIÓN DEL MISMO

y así mismo declaran que ceden y transfieren, sin limitación, a
favor de la sociedad MONSANTO TECHNOLOGY LLC constituida y
existente de conformidad con las leyes del Estado de Delaware,
Estados Unidos de América, y domiciliada en el 800 North Lindbergh
Boulevard, St. Louis, Missouri 63167, Estados Unidos de América
todos los derechos inherentes a dicha invención y que dicha
compañía queda facultada para solicitar en su nombre la patente de
invención correspondiente en la República de Colombia.

Dado y firmado hoy _____

en _____

Firma Ilegible

6/29/02

Firma
Scott A. Huber

Firma

Firma Ilegible 2 de julio de 2002
Firma
Dennis R. Hoerner, Jr.
Presidente y Asesor de Propiedad Intelectual

AUTENTICACIÓN NOTARIAL (INDIVIDUO) del 24 de junio de 2002 expedida por Notario Público del Estado de Missouri en donde se autentica la firma anterior de Scott A. Huber.

Notario Público Firma Ilegible

Sello:

MICHELLE LUDWIG
Condado de San Louis
Mí Comisión Vence 13 de Julio de 2005

Aparece formato de Apostille sin diligenciar

Apostilla

(Convention de La Haye du 5 octobre 1961)

1. País: Estados Unidos de América
Este documento público
2. ha sido firmado por MICHELLE LUDWIG
3. actuando en capacidad de NOTARIO PÚBLICO
4. lleva el sello/estampilla de MICHELLE LUDWIG - NOTARIO PÚBLICO -ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri
6. El 17 de JULIO de 2002

7. por Matt Blunt, Secretario de Estado, Estado de Missouri

8. No. 76728

Diego F. Vargas
DIEGO F. VARGAS Z.
TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLES - INGLES - ESPAÑOL
RES. 0001 DE ENERO 4 DE 1999
MINISTERIO DE JUSTICIA

9. Sello/estampilla:

10. Firma

SELO DEL SECRETARIO DE ESTADO

Matt Blunt

MISSOURI

ESTADO DE MISSOURI §
§ SS.
CONDADO DE SAN LOUIS §

2002 JUL 2 12 32 PM
SAN LOUIS, MO

ANTE Mí, la autoridad suscrita, hoy 2 de julio de 2002, personalmente compareció Dennis R. Hoerner Jr., quien conozco ser la persona cuyo nombre se suscribe en el anterior instrumento a nombre de Monsanto Technology LLC, y reconoció ante mí que él está debidamente autorizado a firmar solo dicha cesión a nombre de dicha compañía.

Firma Ilegible

Notario

"SELLO NOTARIAL"
Norma R. Pope, Notario Público
Condado de San Louis, Estado de Missouri
Mí Comisión Vence 4/10/2005

Ap stilla

(Convention de La Haye du 5 octobre 1961)

COMO NOTARIO SEXTO DEL CIRCULO DE SANTA FE DE BOGOTA, HAY QUE CONSTAR QUE LA(S) ANTERIOR(ES) FIRMADO(S) PRESENTA(S) SON:

A. Verges

IDENTIFICADOS CON C.C. No.(s)

ES(SON) AUTENTICAS(S)

SANTA FE DE BOGOTA, 12 AUG 2002



[Handwritten signature]

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 35

[Signature]
JEFE DE LEGALIZACIONES
SANTA FE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493902

Diego Verges
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

1. País: Estados Unidos de América

Este documento público

2. ha sido firmado por NORMA R. POPE

3. actuando en capacidad de NOTARIO PÚBLICO

4. lleva el sello/estampilla de NORMA A. POPE - NOTARIO PÚBLICO - ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri

6. El 17 de JULIO de 2002

7. por Matt Blunt, Secretario de Estado, Estado de Missouri

8. No. 76726

9. Sello/estampilla:

10. Firma

SELLO DEL SECRETARIO DE ESTADO

MISSOURI

Matt Blunt

Es traducción fiel y completa de la cual se deja copia en esta oficina para su confrontación.

DVZ//ID-3590/WPCL002G/COLO 12746-807-002-1

Diego F. Vargas
DIEGO F. VARGAS Z.
TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLÉS - INGLÉS - ESPAÑOL
RES. 0001 DE ENERO 4 DE 1999
MINISTERIO DE JUSTICIA

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 35

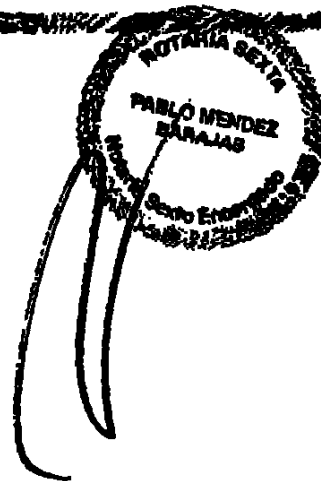
BBB
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493903

Diego Vargas
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

COMO NOTARIO SEXTO DEL CIRCULO DE SANTAFE DE
BOGOTA, HAY QUE CONSTAR QUE LA(S) ANTERIOR(ES) FIR-
MA(S) QUE ESTAN EN ESTE DOCUMENTO SON
Diego Vargas
IDENTIFICADOS CON C.C. No.(s)
ES(SON) AUTENTICA(S)
SANTAFE DE BOGOTA, 12 AUG 2002



Apostille

(Convention de La Haye du 5 octobre 1961)

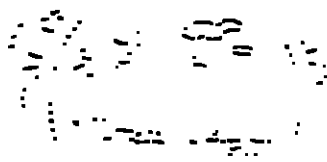
231

1. Country: United States of America

- This public document
2. has been signed by MICHELLE LUDWIG
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of MICHELLE LUDWIG-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76727
9. Seal/Stamp
10. Signature:



Matt Blunt

Apostille

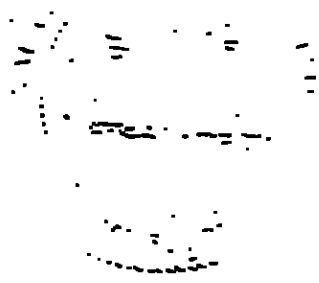
(Convention de La Haye du 5 octobre 1961)

1. Country: United States of America

- This public document
2. has been signed by NORMA R. POPE
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of NORMA R. POPE-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76724
9. Seal/Stamp
10. Signature:



Matt Blunt

CAVELIERABOGADOS
EDIFICIO 81810
CARRERA 4a N° 72-35
BOGOTÁ 4 - COLOMBIA

356

233

CESSION	ASSIGNMENT
Yo, (nosotros) abajo firmante (s)	I (we), the undersigned
Scott A. Huber, James K. Roberts, Zachary W. Shappley, y Sean Doherty,	Scott A. Huber, James K. Roberts, Zachary W. Shappley, and Sean Doherty,
domiciliado(s) en 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South, Collierville, TN 38017 y 1446 Rankin, Richmond Heights, MO 63117, Estados Unidos de América, respectivamente,	of 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South, Collierville, TN 38017 and 1446 Rankin, Richmond Heights, MO 63117, United States of America, respectively,
declaro(amos) bajo juramento ser único(s) y verdadero(s) inventor(es) de la invención:	state(s) under oath to be sole and true inventor(s) of the invention:
PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES Y METODOS PARA DETECCIÓN DEL MISMO	COTTON EVENT MON15985 AND COMPOSITIONS AND METHODS FOR DETECTION THEREOF
declaro(amos) así mismo que cada(amos) y transfero(amos), sin limitación, a favor de la sociedad MONSANTO TECHNOLOGY LLC	state as well, that each, without reservations or limitations in favor of MONSANTO TECHNOLOGY LLC
constituida y existente de conformidad con las leyes del estado de Delaware, Estados Unidos de América	a corporation constituted and existing in conformity with the laws of the state of Delaware, United States of America
domiciliada en 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, Estados Unidos de América	located in 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, United States of America
todos los derechos inherentes a dicha invención y que la citada Compañía queda facultada para solicitar en su nombre la patente de invención correspondiente en la República de Colombia.	all rights inherent to said invention and that the above mentioned company hereby is enabled to apply, in its name, for the corresponding patent application, in the Republic of Colombia

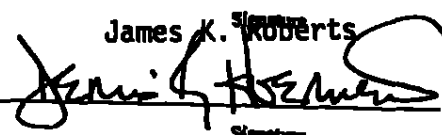
Dado y firmado hoy/ Given and signed this _____

en/In _____


June 25 2002

Firma

James K. Roberts


2 July 2002

Firma

Signature

Dennis R. Hoerner, Jr., President
Intellectual Property Counsel

STATE OF MISSOURI

§

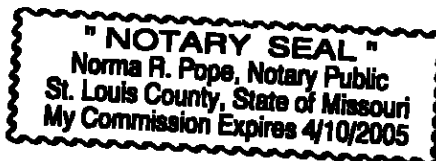
§ ss.

COUNTY OF ST. LOUIS

§

BEFORE ME, the undersigned authority, on this 9th day of July, 2002, personally appeared Dennis R. Hoerner, Jr., known to me to be the person whose name is subscribed to the foregoing instrument on behalf of Monsanto Technology LLC, and acknowledged to me that he is authorized to validly sign alone such an assignment on behalf of said company.


Notary



AUTENTICACION NOTARIAL (INDIVIDUO)

A los 26 día del mes de junio de
2002 ante mí, Notario Público de _____
compareció(ron) personalmente James K. Roberts

a quien(es) doy fe de conocer y me consta que es(son) la(s) persona(s)
firmante(s) del documento que precede, y quien(es) tiene(n) capacidad
legal para otorgarlo.

NOTARIAL AUTHENTICATION (INDIVIDUAL)

X
On this 25th day of June 2002
before me, a Notary Public of St. Louis County
personally appeared James K. Roberts

to me known, and known to me to be the person(s) who executed that
foregoing document, and who has (have) legal capacity to execute it.

Notario Público/Notary Public

Michelle Ludwig



APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country:
This public document
2. has been signed by
3. acting in the capacity of
4. bears the seal/stamp of

Certified

5. at 6. the
7. by
8. No.
9. Seal/stamp 10. Signature

.....

TRADUCCIÓN OFICIAL No 09.08/02e realizada por Diego Vargas,
Traductor Oficial, Resolución No. 0001 de 1999 del Ministerio de
Justicia y registrado ante el Ministerio de Relaciones Exteriores
de Colombia.

A continuación se traducen los sellos y legalizaciones de un
documento de Cesión escrito en inglés y español por medio del cual
Scott A. Huber, James K. Roberts, Zachary W. Shappley y Sean
Doherty, domiciliados en el 681 Hidden Path, Apt. D, Creve Coeur,
MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597
Waterford Cove South, Collierville, TN 38017 y 1446 Rankin,
Richmond Heights, MO 63117, Estados Unidos de América,
respectivamente, declaran bajo juramento ser únicos y verdaderos
inventores de la invención:

PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES Y MÉTODOS PARA
DETECCIÓN DEL MISMO

y así mismo declaran que ceden y transfieren, sin limitación, a
favor de la sociedad MONSANTO TECHNOLOGY LLC constituida y
existente de conformidad con las leyes del Estado de Delaware,
Estados Unidos de América, y domiciliada en el 800 North Lindbergh
Boulevard, St. Louis, Missouri 63167, Estados Unidos de América
todos los derechos inherentes a dicha invención y que dicha
compañía queda facultada para solicitar en su nombre la patente de
invención correspondiente en la República de Colombia.

Dado y firmado hoy _____

en _____

Firma Ilegible Junio 25 de 2002

Firma

James K. Roberts

Firma

Firma Ilegible

2 de julio de 2002

Firma

Firma

Dennis R. Hoerner, Jr.

Presidente y Asesor de Propiedad Intelectual

AUTENTICACIÓN NOTARIAL (INDIVIDUO) del 25 de junio de 2002 expedida por Notario Público del Estado de Missouri en donde se autentica la firma anterior de James K. Roberts.

Notario Público Firma Ilegible

Sello:

MICHELLE LUDWIG
Condado de San Louis
Mí Comisión Vence 13 de Julio de 2005

Aparece formato de Apostille sin diligenciar

Apostilla

(Convention de La Haye du 5 octubre 1961)

1. País: Estados Unidos de América

Este documento público

2. ha sido firmado por MICHELLE LUDWIG

3. actuando en capacidad de NOTARIO PÚBLICO

4. lleva el sello/estampilla de MICHELLE LUDWIG - NOTARIO PÚBLICO -ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri

6. El 17 de JULIO de 2002

7. por Matt Blunt, Secretario de Estado, Estado de Missouri

8. No. 76727

Diego F. Vargas
DIEGO F. VARGAS Z.

TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLÉS - INGLÉS - ESPAÑOL

RES. 0001 DE ENERO 4 DE 1999

MINISTERIO DE JUSTICIA

9. Sello/estampilla:

10. Firma

SELLO DEL SECRETARIO DE ESTADO

Matt Blunt

MISSOURI

ESTADO DE MISSOURI §
§ ss.
CONDADO DE SAN LOUIS §

ANTE Mí, la autoridad suscrita, hoy 2 de Julio de 2002, personalmente compareció Dennis R. Hoerner Jr., quien conozco ser la persona cuyo nombre se suscribe en el anterior instrumento a nombre de Monsanto Technology LLC, y reconoció ante mí que él está debidamente autorizado a firmar solo dicha cesión a nombre de dicha compañía.

Firma Ilegible

Notario

"SELLO NOTARIAL"

Norma R. Pope, Notario Público
Condado de San Louis, Estado de Missouri
Mí Comisión Vence 4/10/2005

Ap stilla

(Convention de La Haye du 5 octobre 1961)

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 36

Pedro
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493904

Pedro Vargas
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

COMO NOTARIO SEXTO DEL CIRCULO DE SANTA FE DE
BOGOTA, M. D. CONSTAR QUE LA(S) ANTERIORES, FIR-
MA(S) PLUMAS

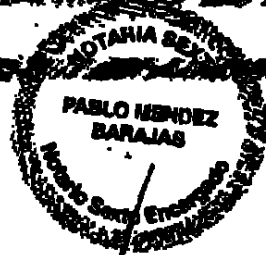
Pedro
Pedro Vargas

NOTARIO SEXTO CO. C. No.(s)

Ed.(s) AUTENTICAS(S)

SANTA FE DE BOGOTA.

12 AUG-2002



1. País: Estados Unidos de América

239

Este documento público

2. ha sido firmado por NORMA R. POPE

3. actuando en capacidad de NOTARIO PÚBLICO

4. lleva el sello/estampilla de NORMA A. POPE - NOTARIO PÚBLICO - ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri

6. El 17 de JULIO de 2002

7. por Matt Blunt, Secretario de Estado, Estado de Missouri

8. No. 76724

9. Sello/estampilla:

10. Firma

SELLO DEL SECRETARIO DE ESTADO

MISSOURI

Matt Blunt

Es traducción fiel y completa de la cual se deja copia en esta oficina para su confrontación.

DVZ//ID-3591/WPCL002G/COLO 12746-807-002-1

Diego F. Vargas
DIEGO F. VARGAS
TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLÉS - INGLÉS - ESPAÑOL
RES. 0001 DE ENERO 4 DE 1999
MINISTERIO DE JUSTICIA

MINISTERIO DE RELACIONES
EXTERIORES

493905

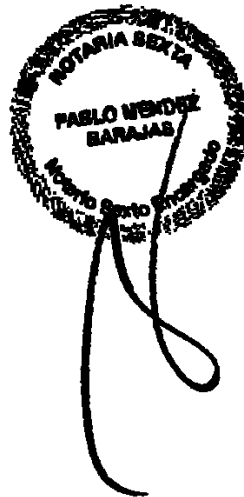
Diego Vargas
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 36

llb
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

COMO NOTARIO SEXTO DEL CIRCULO DE SANTA FE DE BOGOTA, HAGO CONSTAR QUE LA(S) ANTERIORES) FIRMA(S) PUESTA(S) EN
[Signature]
IDENTIFICADOS CON C.C. No.(s)
ES(SON) AUTENTICA(S)
SANTA FE DE BOGOTA, 12 AUG 2002



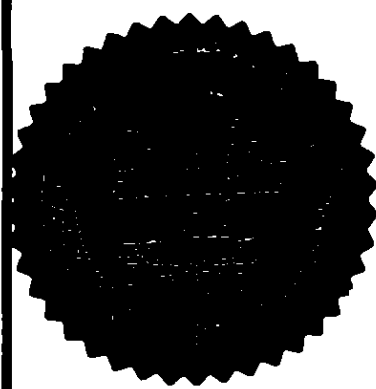
APOSTILLE

(Convention de La Haye du 5 octobre 1961)

- 1. Country: United States of America
- 2. This public document has been signed by JAYNE S. CRESON
- 3. acting in the capacity of COUNTY CLERK OF SHELBY COUNTY, TENNESSEE
- 4. bears the seal/stamp of COUNTY SEAL OF SHELBY COUNTY, TENNESSEE

Certified

- 5. at Nashville, Tennessee
- 6. on Tuesday, July 30, 2002
- 7. by RILEY C. DARNELL, SECRETARY OF STATE, STATE OF TENNESSEE
- 8. No. 02-6177



10. Signature

Riley C. Darnell
dl

Apostille

(Convention de La Haye du 5 octobre 1961)

241

1. Country: United States of America

This public document

2. has been signed by NORMA R. POPE
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of NORMA R. POPE-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76723
9. Seal/Stamp
10. Signature:

Matt Blunt

CAVELIER

ABOGADOS
EDIFICIO 81810
CARRERA 4a N° 72-38
BOGOTÁ, S. COLOMBIA

356

242

CESION**ASSIGNMENT**

Yo, (nosotros) abajo firmante (s)

I (we), the undersigned

Scott A. Huber, James K. Roberts, Zachary W. Shappley, y Sean Doherty,

Scott A. Huber, James K. Roberts, Zachary W. Shappley, and Sean Doherty,

domiciliado(s) en 681 Hidden Path, Apt. D, Creve Coeur, MO 63141,
15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove
South, Collierville, TN 38017 y 1446 Rambla, Richmond Heights, MO
63117, Estados Unidos de América, respectivamente,

of 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover
Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South,
Collierville, TN 38017 and 1446 Rambla, Richmond Heights, MO 63117,
United States of America, respectively,

declaro(amos) bajo juramento ser único(s) y verdadero(s) inventor(es) de
la invención:

state(s) under oath to be sole and true inventor(s) of the invention:

PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES
Y METODOS PARA DETECCIÓN DEL MISMO

COTTON EVENT MON15985 AND COMPOSITIONS AND
METHODS FOR DETECTION THEREOF

declaro(amos) así mismo que cede(amos) y transfiero(amos), sin
limitación, a favor de la sociedad MONSANTO TECHNOLOGY LLC

state as well, that assign, without reservations or limitations in favour of
MONSANTO TECHNOLOGY LLC

constituida y existente de conformidad con las leyes del estado de
Delaware, Estados Unidos de América

a corporation constituted and existing in conformity with the laws of the
state of Delaware, United States of America

domiciliada en 800 North Lindbergh Boulevard, St. Louis, Missouri
63167, Estados Unidos de América

domiciled in 800 North Lindbergh Boulevard, St. Louis, Missouri 63167,
United States of America

todos los derechos inherentes a dicha invención y que la citada Compañía
queda facultada para solicitar en su nombre la patente de invención
correspondiente en la República de Colombia.

all rights inherent to said invention and that the above mentioned
company hereby is enabled to apply, in its name, for the corresponding
patent application, in the Republic of Colombia

Dado y firmado hoy/ Given and signed this _____

ca/in _____

Firma _____

Firma _____

Signature _____

Zachary W. Shappley

Dennis R. Hoerner, Jr.

President & Intellectual Property Counsel

6-28-02

2 July 2002

STATE OF MISSOURI

§

§ ss.

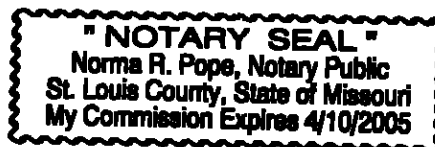
COUNTY OF ST. LOUIS

§

BEFORE ME, the undersigned authority, on this 2nd day of July, 2002, personally appeared Dennis R. Hoerner, Jr., known to me to be the person whose name is subscribed to the foregoing instrument on behalf of Monsanto Technology LLC, and acknowledged to me that he is authorized to validly sign alone such an assignment on behalf of said company.



Notary



STATE OF TENNESSEE

SHELBY COUNTY

CLERK'S OFFICE

244

JAYNE S. CRESON, Clerk of said County do hereby certify

that TAMARA SWAIN

before whom the annexed instrument was taken, and whose name is subscribed in the certificate of proof thereof, was, at the time of taking the same, a Notary Public in and for said county, duly sworn and acting as such, and authorized to take the same, and was also authorized to take acknowledgments to deeds or conveyances of land within the state aforesaid; and further, that I am well acquainted with his handwriting, and verily believe the signature thereto to be his genuine signature; and further, that the annexed instrument is executed and acknowledged according to the laws of the State of Tennessee.

In testimony whereof, I have hereunto set my hand and affixed the seal of said County, at Memphis, Tennessee, this

25TH

day of

JULY

2002

Jayne S. Creson

Clerk

FORMS CONTROL 5-502826-T

AUTENTICACION NOTARIAL (INDIVIDUO)

A los 28 días del mes de Junio de 2002, ante mí, Notario Público de Tennessee, compareció(en) personalmente Zachary W. Shappley

a quien(es) doy fe de conocer y me consta que es(son) la(s) persona(s) firmante(s) del documento que precede, y quien(es) tiene(n) capacidad legal para otorgarlo.

NOTARIAL AUTHENTICATION (INDIVIDUAL)

On this 28 day of June, 2002, before me, a Notary Public of Tennessee, personally appeared Zachary W. Shappley

to me known, and known to me to be the person(s) who executed the foregoing document, and who has (have) legal capacity to execute it.

Notario Público/Notary Public

[Signature]

My Commission Expires March 5, 2005

APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country:
This public document
2. has been signed by
3. acting in the capacity of
4. bears the seal/stamp of

Certified

5. at 6. the
7. by
8. No.
9. Seal/stamp 10. Signature

.....

TRADUCCIÓN OFICIAL No 09.08/02b realizada por Diego Vargas,
Traductor Oficial, Resolución No. 0001 de 1999 del Ministerio de
Justicia y registrado ante el Ministerio de Relaciones Exteriores
de Colombia.

A continuación se traducen los sellos y legalizaciones de un
documento de Cesión escrito en inglés y español por medio del cual
Scott A. Huber, James K. Roberts, Zachary W. Shappley y Sean
Doherty, domiciliados en el 681 Hidden Path, Apt. D, Creve Coeur,
MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597
Waterford Cove South, Collierville, TN 38017 y 1446 Rankin,
Richmond Heights, MO 63117, Estados Unidos de América,
respectivamente, declaran bajo juramento ser únicos y verdaderos
inventores de la invención:

PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES Y MÉTODOS PARA
DETECCIÓN DEL MISMO

y así mismo declaran que ceden y transfieren, sin limitación, a
favor de la sociedad MONSANTO TECHNOLOGY LLC constituida y
existente de conformidad con las leyes del Estado de Delaware,
Estados Unidos de América, y domiciliada en el 800 North Lindbergh
Boulevard, St. Louis, Missouri 63167, Estados Unidos de América
todos los derechos inherentes a dicha invención y que dicha
compañía queda facultada para solicitar en su nombre la patente de
invención correspondiente en la República de Colombia.

Dado y firmado hoy_____

en _____

_____ Firma Ilegible 6-28-02 _____

Firma

Zachary W. Shappley

Firma

247

Firma Ilegible Julio 2 de 2002
Firma Firma

Dennis R. Hoerner, Jr.
Presidente y Asesor de Propiedad Intelectual

AUTENTICACIÓN NOTARIAL (INDIVIDUO) del 28 de junio de 2002 expedida por Notario Público del Estado de Tennessee en donde se autentica la firma anterior de Zachary W. Shappley.

Notario Público Firma Ilegible

Mí Comisión Vence Marzo 5 de 2005

Aparece formato de Apostille sin diligenciar

ESTADO DE TENNESSEE
CONDADO DE SHELBY
OFICINA DEL SECRETARIO

JAYNE S. CRESON, Secretario de dicho Condado por el presente certifico que TAMARA SWAIN

ante quién el documento anexo fue tomado, y cuyo nombre está suscrito en el certificado de prueba del mismo, era, al momento de tomar el mismo, un Notario Público en y para dicho condado, debidamente juramentado y ejerciendo como tal y autorizado para tomar el mismo y así mismo estaba autorizado para tomar reconocimientos de escrituras o transferencias de tierras dentro de dicho estado; y adicionalmente que estoy bien familiarizado con su escritura y creo relamente que la firma en el mismo es su firma auténtica; y adicionalmente que el anexo instrumento ha sido suscrito y reconocido en concordancia con las leyes del Estado de Tennessee.

En testimonio de lo cual, he suscrito el presente y fijado el
sello de dicho Condado en Memphis, Tennessee, hoy

25 de julio de 2002

Firma Ilegible Secretario.

APOSTILLA

(Convention de La Haye du 5 octobre 1961)

- 1. País: Estados Unidos de América
- 2. Este documento público ha sido firmado por JAYNE S. CRESON
- 3. actuando en calidad de SECRETARIO DE CONDADO DEL CONDADO DE SHELBY, TENNESSEE
- 4. lleva sello/estampilla de SECRETARIO DE CONDADO DEL CONDADO DE SHELBY, TENNESSEE

Certificado

- 5. En Nashville, Tennessee
- 6. El Martes, 30 de julio de 2002
- 7. por RILEY C. DARNELL, SECRETARIO DE ESTADO, ESTADO DE TENNESSEE

- 8. No. 02-6177

Diego F. Vargas
DIEGO F. VARGAS Z.
TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLES - INGLES - ESPAÑOL
RES. 0001 DE ENERO 4 DE 1992
MINISTERIO DE JUSTICIA
10. Firma



Firma Ilegible

COMO NOTARIO SEXTO DEL CIRCULO DE SANTAFE DE BOGOTA, HA CONSTAR QUE LA(S) ANTERIOR(ES) FIRMA(S), PUESTA(S) EN

Diego Vargas

IDENTIFICADOS CON C.C. No.(s)

Es(SON) AUTENTICA(S)

SANTAFE DE BOGOTA, 12 AUG 2002



Diego Vargas

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 34

Diego Vargas
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493898

Diego Vargas
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

ANTE Mí, la autoridad suscrita, hoy 2 de julio de 2002, personalmente compareció Dennis R. Hoerner Jr., quien conozco ser la persona cuyo nombre se suscribe en el anterior instrumento a nombre de Monsanto Technology LLC, y reconoció ante mí que él está debidamente autorizado a firmar solo dicha cesión a nombre de dicha compañía.

Firma Ilegible

Notario

"SELLO NOTARIAL"

Norma R. Pope, Notario Público
Condado de San Louis, Estado de Missouri
Mí Comisión Vence 4/10/2005

Apostilla

(Convention de La Haye du 5 octobre 1961)

1. País: Estados Unidos de América

Este documento público

2. ha sido firmado por NORMA R. POPE
3. actuando en capacidad de NOTARIO PÚBLICO
4. lleva el sello/estampilla de NORMA A. POPE - NOTARIO PÚBLICO - ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri
6. El 17 de JULIO de 2002
7. por Matt Blunt, Secretario de Estado, Estado de Missouri

250

8. No. 76723

9. Sello/estampilla:

10. Firma



Matt Blunt

MISSOURI

Es traducción fiel y completa de la cual se deja copia en esta oficina para su confrontación.

DVZ//ID-3586/WPCL002G/COLO 12746-807-002-1

Diego F. Vargas
DIEGO F. VARGAS Z.
TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLÉS - INGLÉS - ESPAÑOL
RES. 0001 DE ENERO 4 DE 1999
MINISTERIO DE JUSTICIA

1999
12746-807-002-1

97

COMO NOTARIO SEXTO DEL CIRCULO DE SANTAFE DE BOGOTA, HAGO CONSTAR QUE LA(S) ANTERIOR(ES) FIRMA(S) PUESTA(S), P. P.

[Signature]

IDENTIFICADOS CON C.C. NO.(A)

ES(SON) AUTENTICA(S)

SANTAFE DE BOGOTA, 12 AUG 2002

[Circular Stamp: NOTARIO SEXTO DE SANTAFE DE BOGOTA]

NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 31u

[Signature]
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493899

[Signature]
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS

251

Apostille

(Convention de La Haye du 5 octobre 1961)

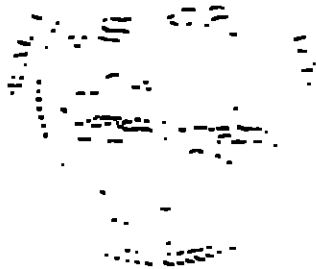
1. Country: United States of America

- This public document
2. has been signed by GRACIE L. WILLIAMS
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of GRACIE L. WILLIAMS-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76729
9. Seal/Stamp
10. Signature:

Comm. 48 (12-2000)



Matt Blunt

252

Apostille

(Convention de La Haye du 5 octobre 1961)

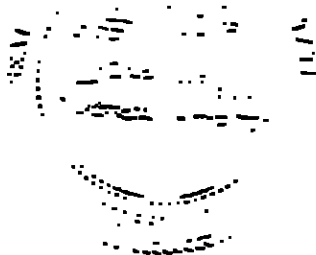
1. Country: United States of America

- This public document
2. has been signed by NORMA R. POPE
3. acting in the capacity of NOTARY PUBLIC
4. bears the seal/stamp of NORMA R. POPE-NOTARY PUBLIC-STATE OF MISSOURI

Certified

5. at Jefferson City, Missouri
6. the 17th. day of JULY, 2002
7. by Matt Blunt, Secretary of State, State of Missouri
8. No. 76725
9. Seal/Stamp
10. Signature:

Comm. 48 (12-2000)



Matt Blunt

CAVELIERABOGADOS
EDIFICIO 8181
CARRERA 46 N° 72-35
BOGOTÁ, D. C. COLOMBIA

356

253

CESION**ASSIGNMENT**

Yo, (nosotros) abajo firmante (s)

I (we), the undersigned

Scott A. Huber, James K. Roberts, Zachary W. Shappley, y Sean Doherty,

Scott A. Huber, James K. Roberts, Zachary W. Shappley, and Sean Doherty,

domiciliado(s) en 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South, Collierville, TN 38017 y 1446 Rankin, Richmond Heights, MO 63117, Estados Unidos de América, respectivamente,

of 681 Hidden Path, Apt. D, Creve Coeur, MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597 Waterford Cove South, Collierville, TN 38017 and 1446 Rankin, Richmond Heights, MO 63117, United States of America, respectively,

declaro(amos) bajo juramento ser único(s) y verdadero(s) inventor(es) de la invención:

state(s) under oath to be sole and true inventor(s) of the invention:

PLANTA EVENTO DE ALGODÓN MON15915 Y COMPOSICIONES Y METODOS PARA DETECCIÓN DEL MISMO

COTTON EVENT MON15915 AND COMPOSITIONS AND METHODS FOR DETECTION THEREOF

declaro(amos) así mismo que cada(amos) y transfero(amos), sin limitación, a favor de la sociedad MONSANTO TECHNOLOGY LLC

state as well, that assign, without reservations or limitations in favour of MONSANTO TECHNOLOGY LLC

constituida y existente de conformidad con las leyes del estado de Delaware, Estados Unidos de América

a corporation constituted and existing in conformity with the laws of the state of Delaware, United States of America

domiciliada en 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, Estados Unidos de América

domiciled in 800 North Lindbergh Boulevard, St. Louis, Missouri 63167, United States of America

todos los derechos inherentes a dicha invención y que la citada Compañía queda facultada para solicitar en su nombre la patente de invención correspondiente en la República de Colombia.

all rights inherent to said invention and that the above mentioned company hereby is enabled to apply, in its name, for the corresponding patent application, in the Republic of Colombia

Dado y firmado hoy/ Given and signed this _____

en/ in _____

X 7/12/02

Firma

Sean C. Shoff

Signature

7/12/02

Firma

Sean Doherty

Signature

Dennis R. Hoerner, Jr.
President & Intellectual Property Counsel

254

STATE OF MISSOURI

§

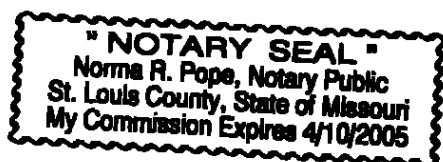
§ ss.

COUNTY OF ST. LOUIS

§

BEFORE ME, the undersigned authority, on this 12th day of July, 2002, personally appeared Dennis R. Hoerner, Jr., known to me to be the person whose name is subscribed to the foregoing instrument on behalf of Monsanto Technology LLC, and acknowledged to me that he is authorized to validly sign alone such an assignment on behalf of said company.

Norma R. Pope
Notary



AUTENTICACION NOTARIAL (INDIVIDUO)

A los 12 días del mes de Julio de 2002 ante mí, Notario Público de compartido(aron) personalmente Sean O'Leary

a quien(es) doy fe de conocer y me consta que es(son) la(s) persona(s) firmante(s) del documento que precede, y quien(es) tiene(n) capacidad legal para otorgarlo.

Notario Público/Notary Public

NOTARIAL AUTHENTICATION (INDIVIDUAL)

On this 12th day of July 2002 before me, a Notary Public of personally appeared Sean O'Leary

to me known, and known to me to be the person(s) who executed the foregoing document, and who has (have) legal capacity to execute it.

Gracie Williams
July, 12, 2002



APOSTILLE

(Convention de La Haye du 5 octobre 1961)

1. Country:
This public document
2. has been signed by
3. acting in the capacity of
4. bears the seal/stamp of

Certified

5. at 6. the
7. by
8. No.
9. Seal/stamp 10. Signature

.....

256
TRADUCCIÓN OFICIAL No 09.08/02c realizada por Diego Vargas,
Traductor Oficial, Resolución No. 0001 de 1999 del Ministerio de
Justicia y registrado ante el Ministerio de Relaciones Exteriores
de Colombia.

A continuación se traducen los sellos y legalizaciones de un
documento de Cesión escrito en inglés y español por medio del cual
Scott A. Huber, James K. Roberts, Zachary W. Shappley y Sean
Doherty, domiciliados en el 681 Hidden Path, Apt. D, Creve Coeur,
MO 63141, 15422 Clover Ridge Dr., Chesterfield, MO 63017, 3597
Waterford Cove South, Collierville, TN 38017 y 1446 Rankin,
Richmond Heights, MO 63117, Estados Unidos de América,
respectivamente, declaran bajo juramento ser únicos y verdaderos
inventores de la invención:

PLANTA EVENTO DE ALGODÓN MON15985 Y COMPOSICIONES Y MÉTODOS PARA
DETECCIÓN DEL MISMO

y así mismo declaran que ceden y transfieren, sin limitación, a
favor de la sociedad MONSANTO TECHNOLOGY LLC constituida y
existente de conformidad con las leyes del Estado de Delaware,
Estados Unidos de América, y domiciliada en el 800 North Lindbergh
Boulevard, St. Louis, Missouri 63167, Estados Unidos de América
todos los derechos inherentes a dicha invención y que dicha
compañía queda facultada para solicitar en su nombre la patente de
invención correspondiente en la República de Colombia.

Dado y firmado hoy _____

en _____

7/12/02

Firma Ilegible

Firma

Sean Doherty

Firma

257

7/12/02

Firma Ilegible

Firma

Firma

Dennis R. Hoerner, Jr.
Presidente y Asesor de Propiedad Intelectual

AUTENTICACIÓN NOTARIAL (INDIVIDUO) del 12 de julio de 2002 expedida por Notario Público del Estado de Missouri en donde se autentica la firma anterior de Sean Doherty.

Notario Público Firma Ilegible

Julio 12 de 2002

Sello:

GRACIE L. WILLIAMS
Condado de San Louis
Mí Comisión Vence 9 de Julio de 2005

Aparece formato de Apostille sin diligenciar

Apostilla

(Convention de La Haye du 5 octubre 1961)

1. País: Estados Unidos de América

Este documento público

2. ha sido firmado por GRACIE L. WILLIAMS

3. actuando en capacidad de NOTARIO PÚBLICO

4. lleva el sello/estampilla de GRACIE L. WILLIAMS - NOTARIO PÚBLICO -ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri

6. El 17 de JULIO de 2002

7. por Matt Blunt, Secretario de Estado, Estado de Missouri

8. No. 76729

Diego F. Vargas
DIEGO F. VARGAS Z.

TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLÉS - INGLÉS - ESPAÑOL

RES. 0001 DE ENERO 4 DE 1999

MINISTERIO DE JUSTICIA

9. Sello/estampilla:

10. Firma

SELLO DEL SECRETARIO DE ESTADO

Matt Blunt

MISSOURI

ESTADO DE MISSOURI

§

§ ss.

CONDADO DE SAN LOUIS

§

ANTE Mí, la autoridad suscrita, hoy 2 de julio de 2002, personalmente compareció Dennis R. Hoerner Jr., quien conozco ser la persona cuyo nombre se suscribe en el anterior instrumento a nombre de Monsanto Technology LLC, y reconoció ante mí que él está debidamente autorizado a firmar solo dicha cesión a nombre de dicha compañía.

Firma Ilegible

Notario

"SELLO NOTARIAL"

Norma R. Pope, Notario Público
Condado de San Louis, Estado de Missouri
Mí Comisión Vence 4/10/2005

Ap stilla

(Convention de La Haye du 5 octobre 1961)

COMO NOTARIO SEXTO DEL CIRCULO DE SANTA FE DE BOGOTA. HAGO CONSTAR QUE LA(S) ANTERIORES FIRMA(S) PUSIERON POR

[Signature]

IDENTIFICADOS CON C.C. No.(s)

Ed(SON) AUTENTICA(S)

SANTA FE DE BOGOTA, 12 AUG 2002



NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 35

[Signature]
JEFE DE LEGALIZACIONES
SANTA FE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493900

Diego Vazquez
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPENA
LAS FUNCIONES INDICADAS

1. País: Estados Unidos de América

Este documento público

2. ha sido firmado por NORMA R. POPE

3. actuando en capacidad de NOTARIO PÚBLICO

4. lleva el sello/estampilla de NORMA A. POPE - NOTARIO PÚBLICO - ESTADO DE MISSOURI

Certificado

5. En Jefferson City, Missouri

6. El 17 de JULIO de 2002

7. por Matt Blunt, Secretario de Estado, Estado de Missouri

8. No. 76725

9. Sello/estampilla:

10. Firma

SELLO DEL SECRETARIO DE ESTADO

MISSOURI

Matt Blunt

Es traducción fiel y completa de la cual se deja copia en esta oficina para su confrontación.

DVZ//ID-3589/WPCL002G/COLO 12746-807-002-1

Diego F. Vargas
DIEGO F. VARGAS Z.
TRADUCTOR E INTERPRETE OFICIAL
ESPAÑOL - INGLÉS - INGLÉS - ESPAÑOL
RES. 0001 DE ENERO 4 DE 1999
MINISTERIO DE JUSTICIA

100

COMO NOTARIO SEXTO DEL CIRCULO DE SANTA FE DE BOGOTA, HAY QUE CONSTAR QUE LAS ANTERIORES FIRMANAS PUES...

[Signature]

NOTARIADOS CON C.C. N.º. (9)

ES CON: AUTENTICA(S)

SANTA FE DE BOGOTA, 12 AUG 2002



NO SE ASUME
RESPONSABILIDAD DEL TEXTO

2002 AUG 12 A 9 35

[Signature]
JEFE DE LEGALIZACIONES
SANTA FE DE BOGOTA D.C.

MINISTERIO DE RELACIONES
EXTERIORES

493901

[Signature]
CUYA FIRMA APARECE EN EL
DOCUMENTO DESEMPEÑA
LAS FUNCIONES INDICADAS



2do. EXAMEN DE FORMA PATENTE DE INVENCION

Folio 163

Radicación no 02-49630

Concepto Técnico no 947

Clasificación Internacional de Patentes: A01H 01/06

Practicado el examen de acuerdo a lo establecido en el Art. 38 de la Decisión 486 se determinó que esta solicitud de Patente de Invención:

- SI ☒ NO ☐ Contiene un resumen de la Invención (Art.26-e).
- SI ☒ NO ☐ Contiene el título o nombre de la invención (Art.27-d).
- SI ☒ NO ☐ Contiene la descripción de la Invención (Art.26-b).
- SI ☒ NO ☐ NO ES APLICABLE ☐ Contiene los dibujos necesarios para comprender la Invención (Art.26-d)
- SI ☒ NO ☐ Contiene una o más reivindicaciones. (Art.26-c).
- SI ☐ NO ☒ NO ES APLICABLE ☐ Han sido desarrollados los productos y/o procedimientos a partir de recursos genéticos o de sus productos derivados de los que cualquiera de los Países Miembros es país de origen. (Art. 26- h).
- SI ☐ NO ☐ NO ES APLICABLE ☒ Los productos y/o procedimientos han sido obtenidos o desarrollados a partir de los conocimientos tradicionales de las comunidades indígenas, afro americanas, o locales de los países miembros, de acuerdo a lo establecido en la Decisión 391 y sus modificaciones y reglamentaciones vigentes.(Art.26-I).
- SI ☒ NO ☐ La invención se refiere a un producto relativo a un material biológico. (Art.26-J).
- SI ☒ NO ☐ NO ES APLICABLE ☐ La prioridad coincide con la materia reivindicada.
- SI ☒ NO ☐ CUMPLE LOS REQUISITOS TÉCNICOS

OBSERVACIONES Y OTROS: SI ☐ NO ☒ ver hoja(s) anexa(s)

EXAMINADOR TÉCNICO
202052

Bogotá D. C., diciembre 19 de 2003.



Folio 185

2020
Bogotá DC

Doctor(a)
EMILIO FERRERO
Apoderado(a)
MONSANTO TECHNOLOGY LLC.

REFERENCIA:	Radicación No.	02-49630
	Trámite	002
	Evento	001
	Actuación	416
	Oficio No.	1486
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en los artículos 26 y 27 de la Decisión 486 de la Comisión de la Comunidad Andina, y en las demás disposiciones vigentes sobre la materia, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial, conforme lo establece el artículo 40 de la misma Decisión.

Cúmplase
Dado en Bogotá DC,

26 DIC. 2003


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

202052