US009683242B2

(12) **United States Patent**
Mason et al.

(10) **Patent No.: US 9,683,242 B2**
(45) **Date of Patent: Jun. 20, 2017**

(54) **HERBICIDE TOLERANT SOYBEAN PLANTS AND METHODS FOR IDENTIFYING SAME**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1199 days.

(21) Appl. No.: **13/511,631**

(22) PCT Filed: **Nov. 23, 2010**

(86) PCT No.: **PCT/US2010/057886**

§ 371 (c)(1), (2), (4) Date: **Aug. 9, 2012**

(87) PCT Pub. No.: **WO2011/063413**

PCT Pub. Date: **May 26, 2011**

(65) **Prior Publication Data**

US 2012/0304331 A1 Nov. 29, 2012

Related U.S. Application Data

(60) Provisional application No. 61/367,251, filed on Jul. 23, 2010, provisional application No. 61/263,707, filed on Nov. 23, 2009.

(30) **Foreign Application Priority Data**

Nov. 23, 2009 (EP) 09014565

(51) **Int. Cl.**

C12Q 1/68	(2006.01)
C12P 19/34	(2006.01)
C07H 21/04	(2006.01)
C12N 15/82	(2006.01)
A01H 5/10	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/8274*** (2013.01); ***A01H 5/10*** (2013.01); ***C07H 21/04*** (2013.01); ***C12N 15/8275*** (2013.01); ***C12Q 1/6895*** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

The invention provides specific transgenic soybean plants, plant material and seeds, characterized in that these products harbor a stack of specific transformation events at specific locations in the soybean genome. Tools are also provided which allow rapid and unequivocal identification of these events in biological samples.

40 Claims, 6 Drawing Sheets

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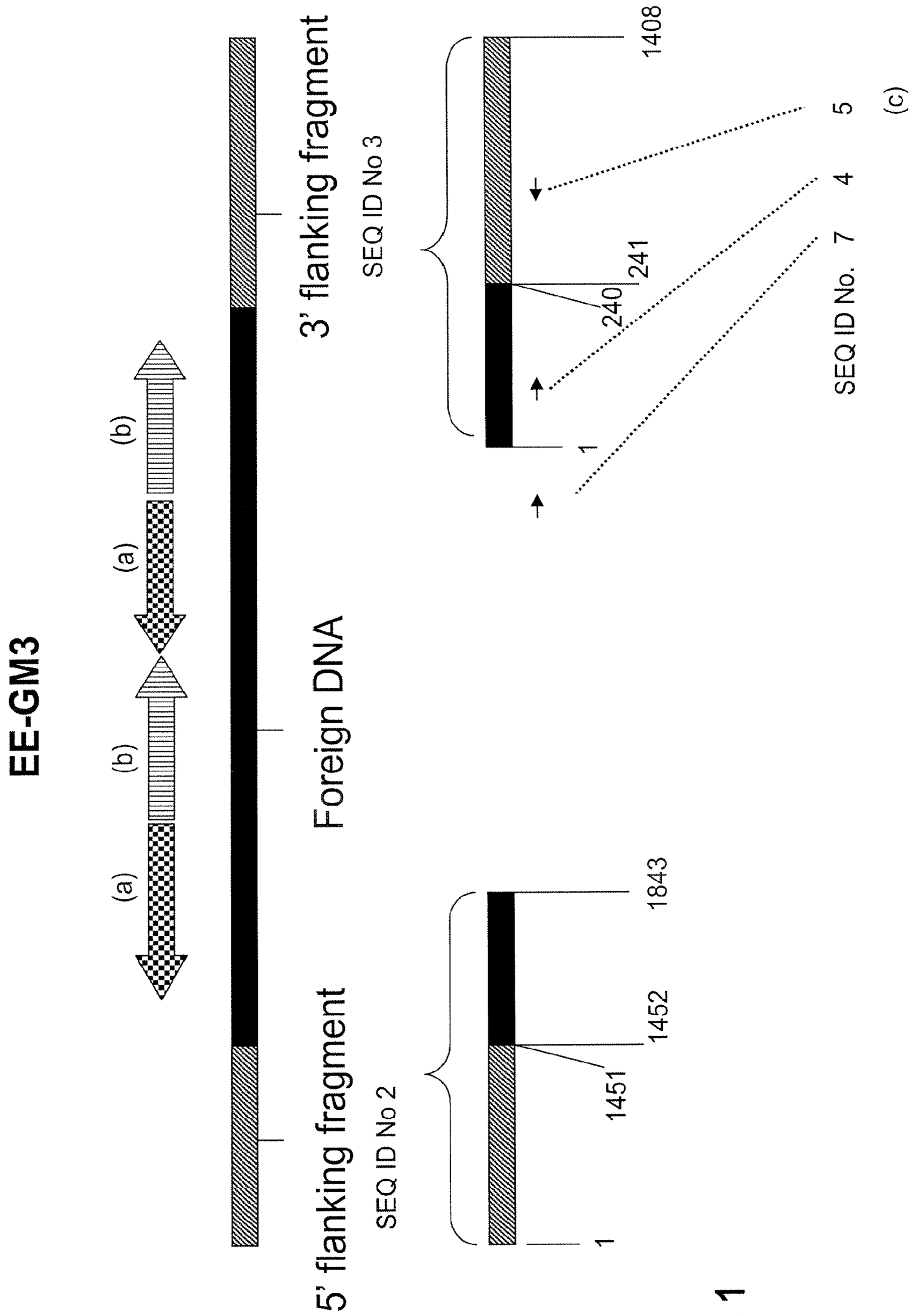
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(a)
(b)
(a)
(b)
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1451
1452
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3' flanking fragment
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1
240
241
1408
SEQ ID No. 7
4
5
(c)

Fig. 1

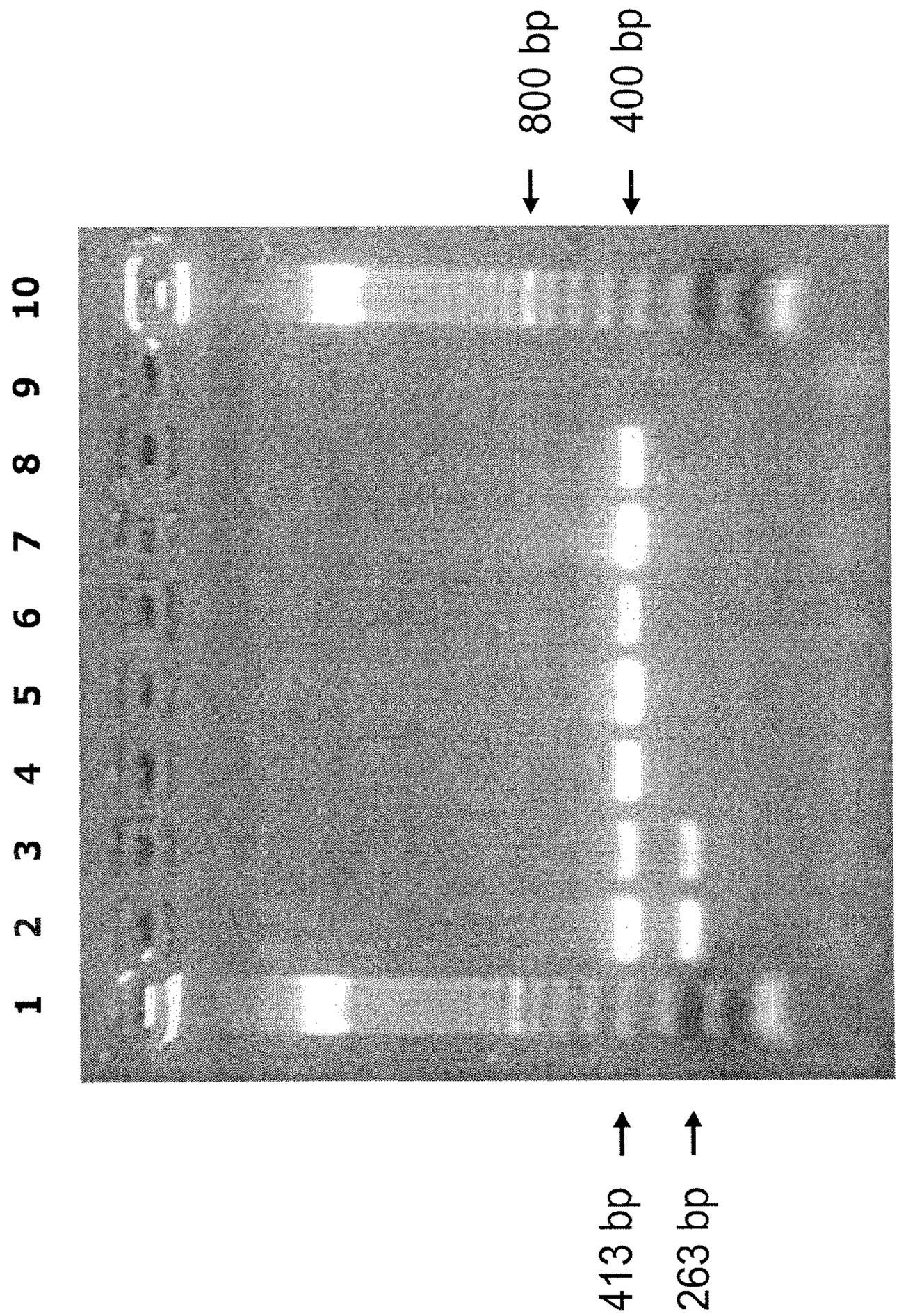


Fig. 2

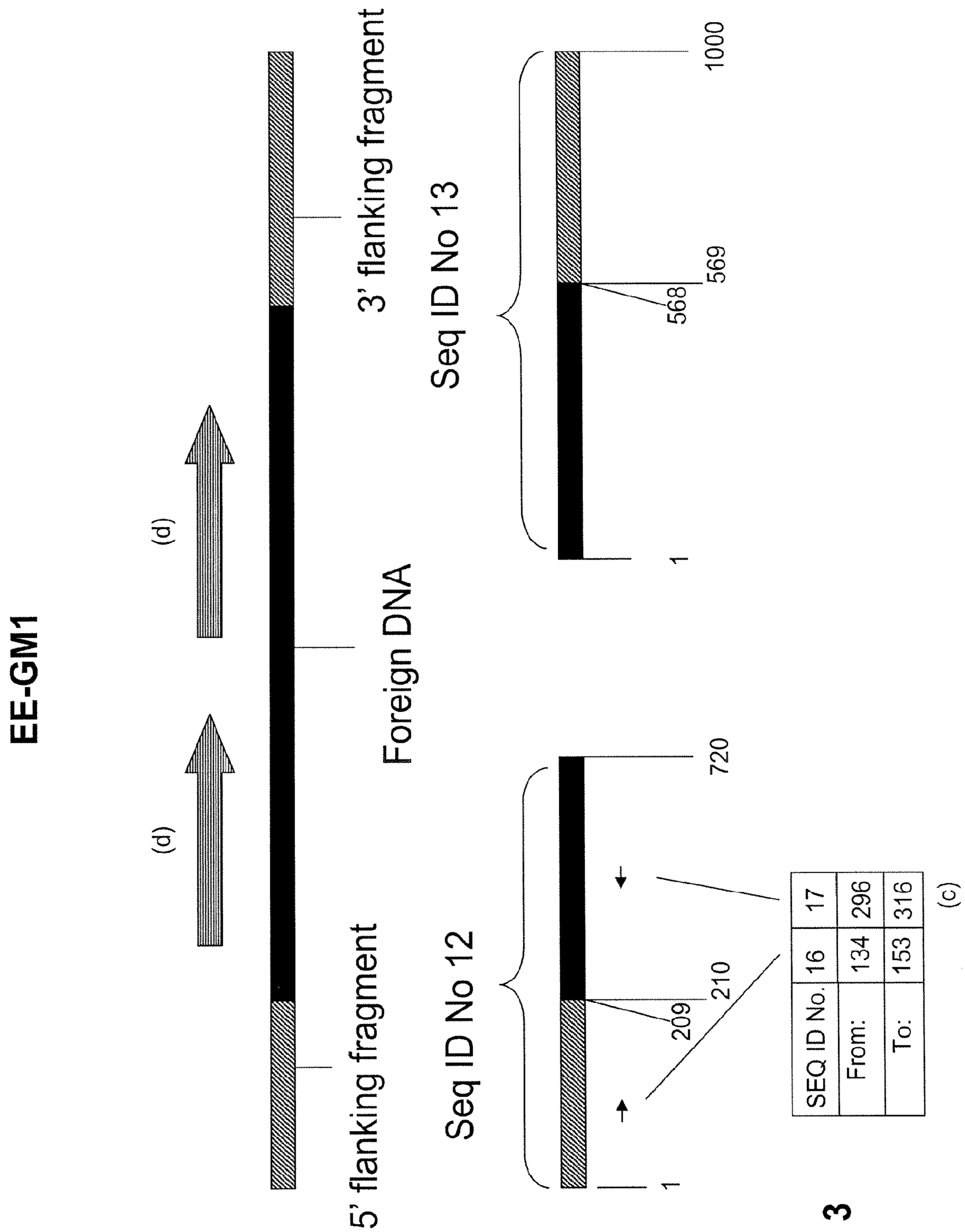
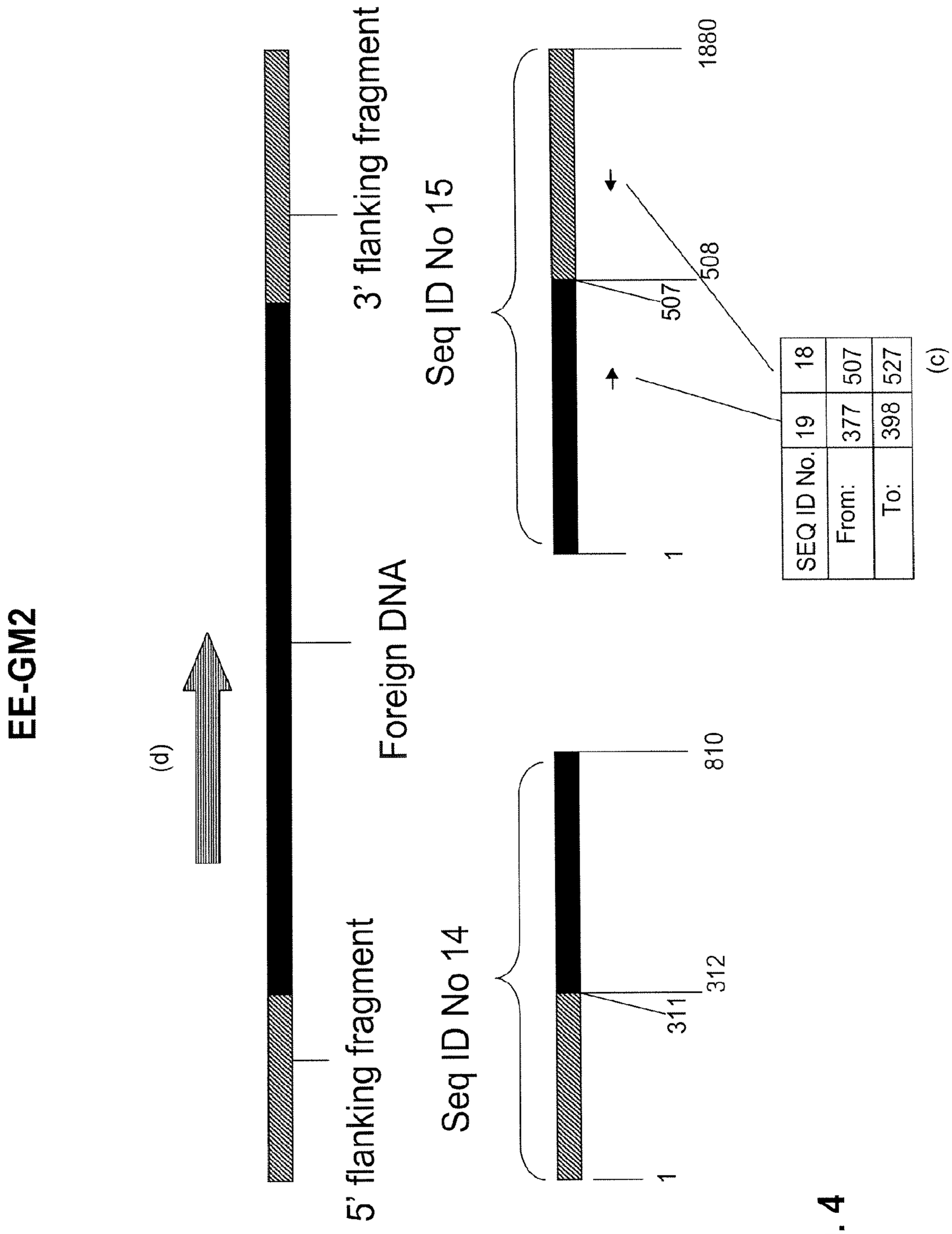

EE-GM1
(d)
(d)
5' flanking fragment
Foreign DNA
3' flanking fragment
Seq ID No 12
Seq ID No 13
1
209
210
720
1
568
569
1000
SEQ ID No. 16 17
From: 134 296
To: 153 316
(c)

Fig. 3



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To:	398	527

Fig. 4

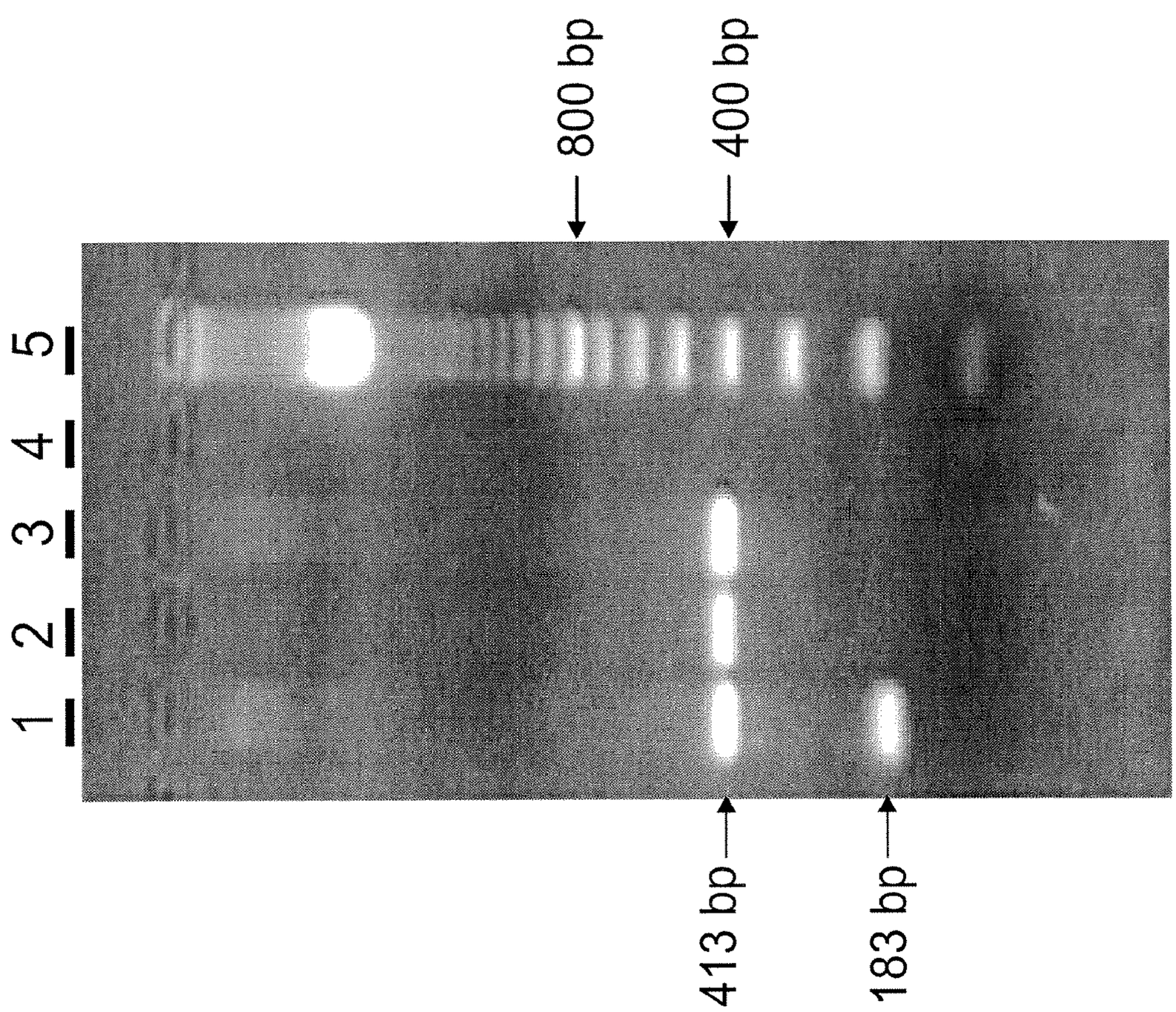

Fig. 5

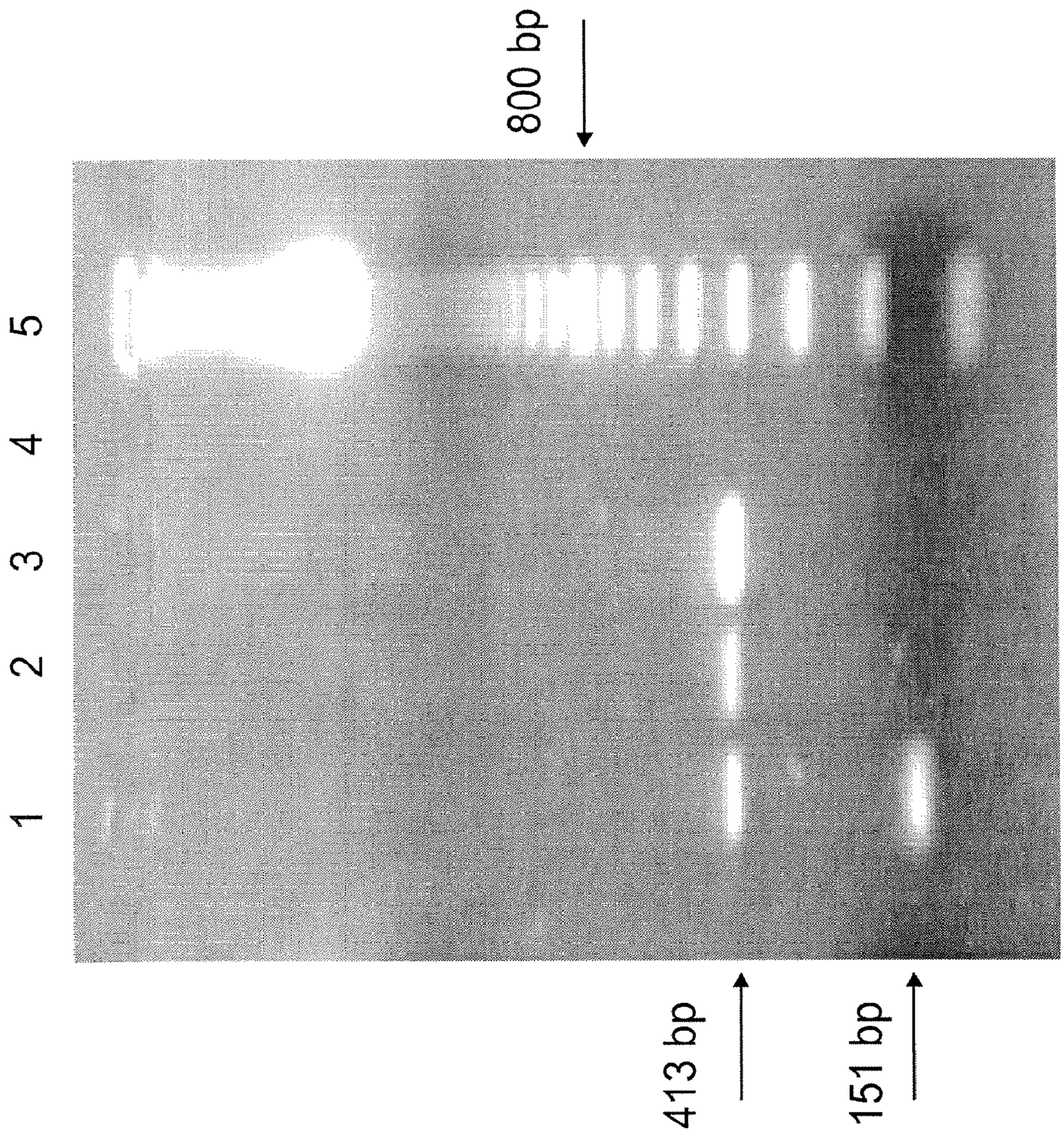

Fig. 6

HERBICIDE TOLERANT SOYBEAN PLANTS AND METHODS FOR IDENTIFYING SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the national phase application of PCT/US2010/057886, filed Nov. 23, 2010, which claims the benefit of EP 09014565.7, filed Nov. 23, 2009, U.S. Provisional Application 61/263,707, filed Nov. 23, 2009 and U.S. Provisional Application 61/367,251 filed Jul. 23, 2010, the contents of each are hereby incorporated by reference in their entirety.

FIELD OF THE INVENTION

This invention relates to transgenic soybean plants, plant material and seeds, characterized by harboring at least two specific transformation events, particularly by the presence of at least two sets of genes encoding proteins that confer herbicide tolerance, each at a specific location in the soybean genome. The soybean plants of the invention combine the herbicide tolerance phenotype with an agronomic performance, genetic stability and functionality in different genetic backgrounds equivalent to the non-transformed soybean line in the absence of herbicide(s). This invention further provides methods and kits for identifying the presence of plant material comprising specifically transformation event EE-GM3 and EE-GM2 or EE-GM1 in biological samples.

BACKGROUND OF THE INVENTION

The phenotypic expression of a transgene in a plant is determined both by the structure of the gene or genes itself and by its or their location in the plant genome. At the same time the presence of the transgenes or "foreign DNA" at different locations in the genome will influence the overall phenotype of the plant in different ways. The agronomically or industrially successful introduction of a commercially interesting trait in a plant by genetic manipulation can be a lengthy procedure dependent on different factors. The actual transformation and regeneration of genetically transformed plants are only the first in a series of selection steps, which include extensive genetic characterization, breeding, and evaluation in field trials, eventually leading to the selection of an elite event.

The unequivocal identification of an elite event is becoming increasingly important in view of discussions on Novel Food/Feed, segregation of GMO and non-GMO products and the identification of proprietary material. Ideally, such identification method is both quick and simple, without the need for an extensive laboratory set-up. Furthermore, the method should provide results that allow unequivocal determination of the elite event without expert interpretation, but which hold up under expert scrutiny if necessary. Specific tools for use in the identification of elite event EE-GM3 and EE-GM1 or EE-GM2 in biological samples are described herein.

In this invention, EE-GM3 has been identified as an elite event from a population of transgenic soybean plants in the development of herbicide tolerant soybean (*Glycine max*) comprising a gene coding for glyphosate tolerance combined with a gene conferring tolerance to 4-hydroxy phenylpyruvate dioxygenase (HPPD) inhibitors, each under control of a plant-expressible promoter.

EE-GM1 and EE-GM2 have previously been identified as elite events from a population of transgenic soybean plants in the development of herbicide tolerant soybean (*Glycine max*) comprising a gene coding for glufosinate tolerance under control of a plant-expressible promoter and are described in WO2006/108674 and WO2006/108675 (both publications herein incorporated by reference).

Soybean plants comprising a herbicide tolerance gene have been disclosed in the art. However, none of the prior art disclosures teach or suggest the present invention.

It is known in the art that getting a commercial herbicide tolerant elite transformation event in soybean plants with acceptable agronomic performance, with no yield drag, and providing sufficient herbicide tolerance, certainly to 3 different classes of herbicides, is by no means straightforward.

Indeed, it has been reported that the first soybean event (event 40-3-2) released on the market with herbicide tolerance, had a significant yield drag compared to (near-) isogenic lines (Elmore et al. (2001) Agron. J. 93:408-412).

Also, Optimum™ GAT™ soybeans (event 356043) were developed to combine tolerance to glyphosate with tolerance to ALS herbicides, but it has been reported that these soybeans were not meeting the standards for glyphosate tolerance by itself (without combination with another glyphosate tolerance soybean event (such as event 40-3-2) (see, e.g., www.bloomberg.com/apps/news?pid=newsarchive&sid=ad4L0hH9MKWE)).

SUMMARY OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention relates to a transgenic soybean plant, or seed, cells or tissues thereof, comprising, stably integrated into its genome, an expression cassette which comprises a herbicide tolerance gene comprising the coding sequence of the 2mEPSPS gene and another herbicide tolerance gene comprising the coding sequence of HPPD-PF W336 (both as described in Example 1.1 herein and as represented in SEQ ID No 1) as well as a second expression cassette comprising a herbicide tolerance gene comprising the coding sequence of a phosphinothricin acetyl transferase, as described in Example 1 herein and as represented in SEQ ID No 11. The transgenic soybean plant, or seed, cells or tissues thereof are tolerant to herbicides based on glyphosate, based on glufosinate, and an HPPD inhibitor herbicide such as isoxaflutole, and, in the absence of herbicide(s), has an agronomic performance which is substantially equivalent to the non-transgenic isogenic line. After application of one or more herbicides to which tolerance is provided, the plant will have a superior agronomic phenotype compared to a non-transgenic plant.

According to the present invention the soybean plant or seed, cells or tissues thereof comprise elite event EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2.

More specifically, the present invention relates to a transgenic soybean plant, seed, cells or tissues thereof, the genomic DNA of which is characterized by the fact that, when analyzed in a PCR Identification Protocol as described herein, using two primers directed to the 5' or 3' flanking region of EE-GM3 and the foreign DNA comprising herbicide tolerance genes in EE-GM-3, respectively, yields a fragment which is specific for EE-GM3 and further when analyzed in a PCR Identification Protocol as described herein, using two primers directed to the 5' or 3' flanking region of EE-GM1 or EE-GM2 and the foreign DNA comprising glufosinate tolerance genes, respectively, yields a fragment which is specific for EE-GM1 or EE-GM2. The primers for detection of EE-GM3 may be directed against the 5' flanking region within SEQ ID NO: 2 and the foreign

DNA comprising herbicide tolerance genes, respectively. The primers for detection of EE-GM3 may also be directed against the 3' flanking region within SEQ ID NO: 3 and the foreign DNA comprising herbicide tolerance genes, respectively, such as the primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID NO: 5 and SEQ ID NO: 4 or SEQ ID No.: 7 respectively, and yield a DNA fragment of between 100 and 800 bp, such as a fragment of about 263 bp or about 706 bp. The primers for detection of EE-GM1 may be directed against the 5' flanking region within SEQ ID NO: 12 and the foreign DNA comprising herbicide tolerance genes, respectively. The primers for detection of EE-GM1 may also be directed against the 3' flanking region within SEQ ID NO: 13 and the foreign DNA comprising herbicide tolerance genes, respectively, such as the primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID NO: 16 and SEQ ID NO: 17 respectively, and yield a DNA fragment of between 100 and 500 bp, such as a fragment of about 183 bp. The primers for detection of EE-GM2 may be directed against the 5' flanking region within SEQ ID NO: 14 and the foreign DNA comprising herbicide tolerance genes, respectively. The primers for detection of EE-GM2 may also be directed against the 3' flanking region within SEQ ID NO: 15 and the foreign DNA comprising herbicide tolerance genes, respectively, such as the primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID NO: 18 and SEQ ID NO: 19 respectively, and yield a DNA fragment of between 100 and 500 bp, such as a fragment of about 151 bp.

Reference seed comprising the elite event EE-GM3 of the invention has been deposited at the NCIMB under accession number NCIMB 41659. Reference seed comprising the elite event EE-GM1 of the invention has been deposited at the NCIMB under accession number NCIMB 41658. Reference seed comprising the elite event EE-GM2 of the invention has been deposited at the NCIMB under accession number NCIMB 41660. Reference seed comprising elite event EE-GM3 and EE-GM1 was deposited at the ATCC under accession number PTA-11041. Reference seed comprising elite event EE-GM3 and EE-GM2 was deposited at the ATCC under accession number PTA-11042.

One embodiment of the invention is seed comprising elite event EE-GM3 (reference seed comprising said event being deposited as NCIMB accession number NCIMB 41659) and further comprising elite event EE-GM1 (reference seed comprising said event being deposited as NCIMB accession number NCIMB 41658), or seed comprising event EE-GM3 and EE-GM1 (reference seed comprising said events being deposited at the ATCC under accession number PTA-11041) which will grow into a soybean plant tolerant to at least three herbicides, particularly tolerant to glyphosate and/or HPPD inhibitors such as isoxaflutole and/or glutamine synthetase inhibitors such as glufosinate.

Another embodiment of the invention is seed comprising elite event EE-GM3 (reference seed comprising said event being deposited as NCIMB accession number NCIMB 41659) and further comprising elite event EE-GM2 (reference seed comprising said event being deposited as NCIMB accession number NCIMB 41660), or seed comprising event EE-GM3 and EE-GM2 (reference seed comprising said events being deposited at the ATCC under accession number PTA-11042), which will grow into a soybean plant tolerant to at least three herbicides, particularly tolerant to glyphosate and/or HPPD inhibitors such as isoxaflutole and/or glutamine synthetase inhibitors such as glufosinate.

The seed of NCIMB deposit number NCIMB 41659, is a seed lot consisting of at least about 95% transgenic seeds, comprising elite event EE-GM3, which will grow into herbicide tolerant plants, whereby the plants are glyphosate and/or isoxaflutole tolerant plants. The seed or progeny seed obtainable or obtained from the deposited seed (e.g., following crossing with other soybean plants with a different genetic background) can be sown and the growing plants can be treated with glyphosate or HPPD inhibitors such as isoxaflutole as described herein to obtain glyphosate or isoxaflutole-tolerant plants, comprising elite event EE-GM3. The seed of NCIMB deposit number NCIMB 41658 and NCIMB 41660 are seed lots consisting of at least about 95% transgenic seeds, comprising elite events EE-GM1 or EE-GM2, respectively, which will grow into herbicide tolerant plants, whereby the plants are glufosinate-tolerant plants. The seed can be sown and the growing plants can be treated with glufosinate as described herein to obtain glufosinate tolerant plants, comprising the elite events EE-GM1 or EE-GM2.

The seed of ATCC deposit number PTA-11041, is a seed lot consisting of at least about 95% transgenic seeds, comprising elite event EE-GM3 and elite event EE-GM1 in homozygous form, which will grow into herbicide tolerant plants, whereby the plants are glyphosate, glufosinate and/or isoxaflutole tolerant plants. The seed or progeny seed obtainable or obtained from the deposited seed (e.g., following crossing with other soybean plants with a different genetic background) can be sown and the growing plants can be treated with glyphosate, glufosinate and/or HPPD inhibitors such as isoxaflutole, as described herein to obtain glyphosate, glufosinate and/or isoxaflutole-tolerant plants, comprising elite event EE-GM3 and EE-GM1.

The seed of ATCC deposit number PTA-11042, is a seed lot consisting of at least about 95% transgenic seeds, comprising elite event EE-GM3 and elite event EE-GM2 in homozygous form, which will grow into herbicide tolerant plants, whereby the plants are glyphosate, glufosinate and/or isoxaflutole tolerant plants. The seed or progeny seed obtainable or obtained from the deposited seed (e.g., following crossing with other soybean plants with a different genetic background) can be sown and the growing plants can be treated with glyphosate, glufosinate and/or HPPD inhibitors such as isoxaflutole, as described herein to obtain glyphosate, glufosinate and/or isoxaflutole-tolerant plants, comprising elite event EE-GM3 and EE-GM2.

The invention further relates to cells, tissues, progeny, and descendants from a plant comprising the elite event EE-GM3 and further comprising elite event EE-GM1, and which may be obtained by growing a plant comprising the elite event EE-GM3 and EE-GM1 deposited at ATCC as PTA-11041, or by growing a plant comprising the elite event EE-GM3 from the seed deposited at the NCIMB having accession number NCIMB 41659 and crossing said plant with a plant comprising elite event EE-GM1 grown from the seed deposited at the NCIMB having accession number NCIMB 41658. The invention also relates to cells, tissues, progeny, and descendants from a plant comprising the elite event EE-GM3 and further comprising elite event EE-GM2, and which may be obtained by growing a plant comprising the elite event EE-GM3 and EE-GM2 deposited at ATCC as PTA-11042, or by growing a plant comprising the elite event EE-GM3 from the seed deposited at the NCIMB having accession number NCIMB 41659 and crossing said plant with a plant comprising elite event EE-GM2 grown from the seed deposited at the NCIMB having accession number NCIMB 41660. The invention further relates to plants obtainable from (such as by propagation of and/or breeding with) a soybean plant or seed as described immediately

above. The invention also relates to soybean plants comprising elite event EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2.

The invention further relates to a method for identifying a transgenic plant, or cells or tissues thereof, comprising elite event EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2, which method is based on identifying the presence of characterizing DNA sequences or amino acids encoded by such DNA sequences in the transgenic plant, cells or tissues. According to a preferred embodiment of the invention, such characterizing DNA sequences are sequences of at least 15 bp, 15 bp, at least 20 bp, 20 bp, or 30 bp or more which comprise the insertion site of the event, i.e., both a part of the inserted foreign DNA comprising herbicide tolerance genes and a part of the soybean genome (either the 5' or 3' flanking region) contiguous therewith, allowing specific identification of the elite event.

The present invention further relates to methods for identifying elite events EE-GM3 and EE-GM1 or elite events EE-GM3 and EE-GM2 in biological samples, which methods are based on primers or probes which specifically recognize the 5' and/or 3' flanking sequence of the foreign DNA comprising the herbicide tolerance genes in EE-GM3 and EE-GMT or EE-GM3 and EE-GM2.

More specifically, the invention relates to a method comprising amplifying two sequences of a nucleic acid present in biological samples, using two polymerase chain reactions each with at least two primers or one polymerase chain reaction with at least four primers, the first primer recognizing the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes comprising the herbicide tolerance genes in EE-GM3, the second primer recognizing a sequence within the foreign DNA comprising herbicide tolerance genes of EE-GM3, the third primer recognizing the 5' or 3' flanking region of foreign DNA comprising the herbicide tolerance genes in EE-GM1 or EE-GM2, and the fourth primer recognizing a sequence within the foreign DNA comprising the herbicide tolerance genes of EE-GM1 or EE-GM2, preferably to obtain two DNA fragments of between 100 and 800 bp. The primers for identifying EE-GM3 may recognize a sequence within the 5' flanking region of EE-GM3 (SEQ ID No. 2, from position 1 to position 1451) or within the 3' flanking region of EE-GM3 (complement of SEQ ID No 3 from position 241 to position 1408) and a sequence within the foreign DNA comprising the herbicide tolerance genes (complement of SEQ ID No 2 from position 1452 to 1843 or SEQ ID No 3 from position 1 to position 240, or SEQ ID No 20 from nucleotide position 1452 to nucleotide position 16638 or its complement), respectively. The primer recognizing the 3' flanking region may comprise the nucleotide sequence of SEQ ID No. 5 and the primer recognizing a sequence within the foreign DNA comprising herbicide tolerance genes may comprise the nucleotide sequence of SEQ ID No. 4 or SEQ ID No.: 7 described herein. The primers for identifying EE-GM1 may recognize a sequence within the 5' flanking region of EE-GM1 (SEQ ID No. 12, from position 1 to position 209) or within the 3' flanking region of EE-GM1 (complement of SEQ ID No 13 from position 569 to position 1000) and a sequence within the foreign DNA comprising herbicide tolerance gene of EE-GM1 (complement of SEQ ID No 12 from position 210 to 720, or SEQ ID No 13 from position 1 to position 568), respectively. The primer recognizing the 5' flanking region of EE-GM1 may comprise the nucleotide sequence of SEQ ID No. 16 and the primer recognizing a sequence within the foreign DNA of EE-GM1 may comprise the nucleotide sequence of SEQ ID No. 17 described herein. The primers for identifying EE-GM2 may recognize a sequence within the 5' flanking region of EE-GM2 (SEQ ID No. 14, from position 1 to position 311) or within the 3' flanking region of EE-GM2 (complement of SEQ ID No 15 from position 508 to position 1880) and a sequence within the foreign DNA comprising herbicide tolerance gene of EE-GM1 (complement of SEQ ID No 14 from position 312 to 810, or SEQ ID No 15 from position 1 to position 507), respectively. The primer recognizing the 3' flanking region of EE-GM2 may comprise the nucleotide sequence of SEQ ID No. 18 and the primer recognizing a sequence within the foreign DNA of EE-GM2 may comprise the nucleotide sequence of SEQ ID No. 19 described herein. The PCR amplification may be done simultaneously or sequentially.

The present invention more specifically relates to a method for identifying elite event EE-GM3 and EE-GM1 in biological samples, which method comprises amplifying at least two sequences of nucleic acids present in a biological sample, using a polymerase chain reaction with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 4 and SEQ ID No. 5, respectively, to obtain a DNA fragment of about 263 bp, or with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 5 and SEQ ID No. 7 respectively, to obtain a DNA fragment of about 706 bp, and a polymerase chain reaction with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 16 and SEQ ID No. 17 respectively, to obtain a DNA fragment of about 183 bp. The two polymerase chain reactions may be performed simultaneously or sequentially.

The present invention more specifically relates to a method for identifying elite event EE-GM3 and EE-GM2 in biological samples, which method comprises amplifying at least two sequences of nucleic acids present in a biological sample, using a polymerase chain reaction with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 4 and SEQ ID No. 5 respectively, to obtain a DNA fragment of about 263 bp, or with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 5 and SEQ ID No. 7 respectively, to obtain a DNA fragment of about 706 bp, and a polymerase chain reaction with two primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 18 and SEQ ID No. 19 respectively, to obtain a DNA fragment of about 151 bp. The two polymerase chain reactions may be performed simultaneously or sequentially.

The present invention further relates to the specific flanking sequences of EE-GM3 described herein in combination with the specific flanking sequences of EE-GM1, which can be used to develop specific identification methods for simultaneous presence of EE-GM3 and EE-GM1 in biological samples. Such combined specific flanking sequences may also be used as reference control material in identification assays. More particularly, the invention relates to the 5' and/or 3' flanking regions of EE-GM3 in combination with the 5' and/or 3' flanking regions of EE-GM1 which can be used for the development of specific primers and probes as further described herein. Also suitable as reference material are nucleic acid molecules, preferably of about 150-850 bp, comprising the sequence which can be amplified by primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 7 and SEQ ID No. 5 or of SEQ ID No. 4 and SEQ ID No. 5 in combination with nucleic acid molecules comprising the sequence which can be amplified by primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 16 and SEQ ID No. 17,

particularly such nucleic acid molecules are obtained using such primers in material comprising EE-GM3 and EE-GM1.

The present invention further also relates to the specific flanking sequences of EE-GM3 described herein in combination with the specific flanking sequence of EE-GM2, which can be used to develop specific identification methods for simultaneous presence of EE-GM3 and EE-GM2 in biological samples. Such combined specific flanking sequences may also be used as reference control material in identification assays. More particularly, the invention relates to the 5' and/or 3' flanking regions of EE-GM3 in combination with the 5' and/or 3' flanking regions of EE-GM2 which can be used for the development of specific primers and probes as further described herein. Also suitable as reference material are nucleic acid molecules, preferably of about 150-850 bp, comprising the sequence which can be amplified by primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 7 and SEQ ID No. 5 or of SEQ ID No. 4 and SEQ ID No. 5 in combination with nucleic acid molecules comprising the sequence which can be amplified by primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 18 and SEQ ID No. 19, particularly such nucleic acid molecules are obtained using such primers in material comprising EE-GM3 and EE-GM2.

The invention further relates to identification methods for the simultaneous presence of EE-GM3 and EE-GM1 in biological samples based on the use of such specific primers or probes. Primers for EE-GM3 detection may comprise, consist or consist essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451 or the complement of the nucleotide sequence of SEQ ID 3 from nucleotide 241 to nucleotide 1408, combined with primers comprising, consisting, or consisting essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 1, such as a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240. Primers for EE-GM1 detection may comprise, consist or consist essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 12 from nucleotide 1 to nucleotide 209 or the complement of the nucleotide sequence of SEQ ID 13 from nucleotide 569 to nucleotide 1000, combined with primers comprising, consisting, or consisting essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 11, such as a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the complement of the nucleotide sequence of SEQ ID No 12 from nucleotide 219 to nucleotide 720 or the nucleotide sequence of SEQ ID No 13 from nucleotide 1 to nucleotide 568. Primers may also comprise these nucleotide sequences located at their extreme 3' end, and further comprise unrelated sequences or sequences derived from the mentioned nucleotide sequences, but comprising mismatches.

The invention further relates to identification methods for the simultaneous presence of EE-GM3 and EE-GM2 in biological samples based on the use of such specific primers or probes. Primers for EE-GM3 detection may comprise, consist or consist essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides as described in the previous paragraph. Primers for EE-GM2 detection may comprise, consist or consist essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 14 from nucleotide 1 to nucleotide 311 or the complement of the nucleotide sequence of SEQ ID 15 from nucleotide 508 to nucleotide 1880, combined with primers comprising, consisting or consisting essentially of a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No 11, such as a nucleotide sequence of 17 to about 200 consecutive nucleotides selected from the complement of the nucleotide sequence of SEQ ID No 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No 15 from nucleotide 1 to nucleotide 507. Primers may also comprise these nucleotide sequences located at their extreme 3' end, and further comprise unrelated sequences or sequences derived from the mentioned nucleotide sequences, but comprising mismatches.

The invention further relates to kits for identifying elite events EE-GM3 and EE-GM1 in biological samples, said kits comprising at least one primer or probe which specifically recognizes the 5' or 3' flanking region of the foreign DNA comprising herbicide tolerance genes in EE-GM3 and at least one primer or probe which specifically recognizes the 5' or 3' flanking region of the foreign DNA comprising herbicide tolerance genes in EE-GM1.

The invention further relates to kits for identifying elite events EE-GM3 and EE-GM2 in biological samples, said kits comprising at least one primer or probe which specifically recognizes the 5' or 3' flanking region of the foreign DNA comprising herbicide tolerance genes in EE-GM3 and at least one primer or probe which specifically recognizes the 5' or 3' flanking region of the foreign DNA comprising a herbicide tolerance gene in EE-GM2.

The kits of the invention may comprise, in addition to a primer which specifically recognizes the 5' or 3' flanking region of EE-GM3 and the 5' or 3' flanking region of EE-GM1, a further primer which specifically recognizes a sequence within the foreign DNA comprising herbicide tolerance genes of EE-GM3 and a further primer which specifically recognizes a sequence within the foreign DNA comprising herbicide tolerance genes of EE-GM1, for use in a PCR Identification Protocol.

The kits of the invention may also comprise, in addition to a primer which specifically recognizes the 5' or 3' flanking region of EE-GM3 and the 5' or 3' flanking region of EE-GM2, a further primer which specifically recognizes a sequence within the foreign DNA comprising herbicide tolerance genes of EE-GM3 and a further primer which specifically recognizes a sequence within the foreign DNA comprising herbicide tolerance genes of EE-GM2, for use in a PCR Identification Protocol.

The printer recognizing the 3' flanking region of EE-GM3 may comprise the nucleotide sequence of SEQ ID No. 5 and the primer recognizing the transgenes or foreign DNA comprising herbicide tolerance genes of EE-GM3 may comprises the nucleotide sequence of SEQ ID No. 4 or 7, or any other primer or primer combination for EE-GM3 detection as described herein, in combination with a primer recognizing the 5' flanking region of EE-GM1 which may comprise the nucleotide sequence of SEQ ID No. 16 and a primer recognizing the transgenes of foreign DNA comprising the herbicide tolerance gene of EE-GM1 may comprise the nucleotide sequence of SEQ ID No 17.

The primer recognizing the 3' flanking region of EE-GM3 may comprise the nucleotide sequence of SEQ ID No. 5 and

the primer recognizing the transgenes or foreign DNA comprising herbicide tolerance genes of EE-GM3 may comprises the nucleotide sequence of SEQ ID No. 4 or 7, or any other primer or primer combination for EE-GM3 detection as described herein, in combination with a primer recognizing the 5' flanking region of EE-GM2 which may comprise the nucleotide sequence of SEQ ID No. 18 and a primer recognizing the transgenes of foreign DNA comprising the herbicide tolerance gene of EE-GM2 may comprise the nucleotide sequence of SEQ ID No 19.

The invention further relates to a kit for identifying elite event EE-GM3 and EE-GM1 in biological samples, said kit comprising PCR primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 5 and SEQ ID No. 4 and PCR primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID 16 and SEQ ID 17 for use in the EE-GM3/EE-GM1 PCR based identification.

The invention further relates to a kit for identifying elite event EE-GM3 and EE-GM2 in biological samples, said kit comprising PCR primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID No. 5 and SEQ ID No. 4 and PCR primers comprising or consisting (essentially) of the nucleotide sequence of SEQ ID 18 and SEQ ID 19 for use in the EE-GM3/EE-GM2 PCR based identification.

The invention also relates to a kit for identifying elite event EE-GM3 and EE-GM1 in biological samples, which kit comprises two specific probes, a first probe comprising or consisting (essentially) of a sequence which corresponds (or is complementary to) a sequence having between 80% and 100% sequence identity with a specific region of EE-GM3 and a second probe comprising or consisting (essentially) of a sequence which corresponds (or is complementary to) a sequence having between 80% and 100% sequence identity with a specific region of EE-GM1. Preferably, the sequence of the first probe corresponds to a specific region comprising part of the 5' or 3' flanking region of EE-GM3 and the second probe corresponds to a specific region comprising part of the 5' or 3' flanking region of EE-GM1. Most preferably the EE-GM3 specific probe comprises or consists (essentially) of (or is complementary to) a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 1441 to 1462 of SEQ ID No 2 or a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 220 to 260 of ID No. 3 and the EE-GM1 specific probe comprises, consists (essentially) of (or is complementary to) a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 199 to 220 of SEQ ID No 12 or a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 558 to 579 of ID No. 13.

The invention also relates to a kit for identifying elite event EE-GM3 and EE-GM2 in biological samples, which kit comprises two specific probes, a first probe comprising or consisting (essentially) of a sequence which corresponds (or is complementary to) a sequence having between 80% and 100% sequence identity with a specific region of EE-GM3 and a second probe comprising or consisting (essentially) of a sequence which corresponds (or is complementary to) a sequence having between 80% and 100% sequence identity with a specific region of EE-GM2. Preferably, the sequence of the first probe corresponds to a specific region comprising part of the 5' or 3' flanking region of EE-GM3 and the second probe corresponds to a specific region comprising part of the 5' or 3' flanking region of EE-GM2. Most preferably the EE-GM3 specific probe comprises or consists (essentially) of (or is complementary to) a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 1441 to 1462 of SEQ ID No 2 or a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 220 to 260 of ID No. 3 and the EE-GM2 specific probe comprises or consists (essentially) of (or is complementary to) a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 301 to 322 of SEQ ID No 14 or a sequence having between 80% and 100% sequence identity to the sequence between nucleotide 497 to 518 of ID No. 15.

According to another aspect of the invention, DNA sequences are disclosed comprising a junction site of the event and sufficient length of polynucleotides of both the soybean genomic DNA and the foreign DNA comprising herbicide tolerance genes (transgene) of each event, so as to be useful as primer or probe for the detection of EE-GM3 and EE-GM1. Such sequences may comprise at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising herbicide tolerance genes (transgene) of EE-GM3 or EE-GM1, at each side of the junction site respectively. Most preferably, such DNA sequences comprise at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising herbicide tolerance genes contiguous with the junction site in SEQ ID NO: 2 or SEQ ID NO: 3 and at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising herbicide tolerance genes contiguous with the junction site in SEQ ID NO: 12 or SEQ ID NO: 13.

According to yet another aspect of the invention, DNA sequences are disclosed comprising a junction site of the event and sufficient length of polynucleotides of both the soybean genomic DNA and the foreign DNA comprising herbicide tolerance genes (transgene) of each event, so as to be useful as primer or probe for the detection of EE-GM3 and EE-GM2. Such sequences may comprise at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising herbicide tolerance genes (transgene) of EE-GM3 or EE-GM2, at each side of the insertion site respectively. Most preferably, such DNA sequences comprise at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising herbicide tolerance genes contiguous with the junction site in SEQ ID NO: 2 or SEQ ID NO: 3 and at least 9 nucleotides of the soybean genomic DNA and a similar number of nucleotides of the foreign DNA comprising herbicide tolerance genes contiguous with the junction site in SEQ ID NO: 14 or SEQ ID NO: 15.

The methods and kits encompassed by the present invention can be used for different purposes such as, but not limited to the following: to identify the presence or determine the (lower) threshold of EE-GM3 and EE-GM1 or of EE-GM3 and EE-GM2 in plants, plant material or in products such as, but not limited to food or feed products (fresh or processed) comprising or derived from plant material; additionally or alternatively, the methods and kits of the present invention can be used to identify transgenic plant material for purposes of segregation between transgenic and non-transgenic material; additionally or alternatively, the methods and kits of the present invention can be used to determine the quality (i.e., percentage pure material) of plant material comprising EE-GM3 and EE-GM1 or EE-GM3 and EE-GM1.

The invention further relates to the 5' and/or 3' flanking regions of EE-GM3 GM3 in combination with specific primers and probes developed from the 5' and/or 3' flanking sequences of EE-GM1 or EE-GM2, as well as to the specific primers and probes developed from the 5' and/or 3' flanking sequences of EE-GM3 in combination with specific primers and probes developed from the 5' and/or 3' flanking sequences of EE-GM1 or EE-GM2.

The invention also relates to genomic DNA obtained from plants comprising elite events EE-GM3 and EE-GM1 or plants comprising elite events EE-GM3 and EE-GM2. Such genomic DNA may be used as reference control material in the identification assays herein described.

Also provided herein is a transgenic herbicide tolerant soybean plant, or cells, parts, seeds or progeny thereof, each comprising at least two elite events, said first elite event comprising a foreign DNA comprising:

i) a first chimeric gene which comprises a modified epsps gene from *Zea mays* encoding a glyphosate tolerant EPSPS enzyme under the control of a plant-expressible promoter, and

ii) a second chimeric gene which comprises a modified hppd gene from *Pseudomonas fluorescens* encoding an HPPD inhibitor herbicide tolerant enzyme under the control of a plant-expressible promoter, and said second elite event comprises a (third) chimeric gene which comprises a modified glufosinate tolerance gene derived from *Streptomyces viridochromogenes* encoding a glufosinate (or phosphinothricin) acetyltransferase enzyme under the control of a plant-expressible promoter.

In one embodiment, said first elite event comprises nucleotides 1 to 1451 of SEQ ID No 2 immediately upstream of and contiguous with said foreign DNA and nucleotides 241 to 1408 of SEQ ID No 3 immediately downstream of and contiguous with said foreign DNA, and said second event comprises nucleotides 1 to 209 of SEQ ID No 12 immediately upstream of and contiguous with said foreign DNA and nucleotides 569 to 1000 of SEQ ID No 13 immediately downstream of and contiguous with said foreign DNA, or said second event comprises nucleotides 1 to 311 of SEQ ID No 14 immediately upstream of and contiguous with said foreign DNA and nucleotides 508 to 1880 of SEQ ID No 15 immediately downstream of and contiguous with said foreign DNA.

In a further embodiment, said elite event is obtainable by breeding with a soybean plant grown from reference seed comprising said events having been deposited at the ATCC under deposit number PTA-11041 or PTA-11042, or obtainable from reference seed comprising said first event having been deposited at NCIMB under accession number NCIMB 41659, and obtainable from reference seed comprising said second event having been deposited at NCIMB under accession number NCIMB 41658 or NCIMB accession number NCIMB 41660.

In another embodiment, the genomic DNA of said soybean plant, or cells, parts, seeds or progeny thereof when analyzed using the elite event identification protocol for said first elite event with two primers comprising the nucleotide sequence of SEQ ID No 4 and SEQ ID No 5 respectively, yields a DNA fragment of about 263 bp or 263 bp, and when analyzed using the elite event identification protocol for said second elite event with two primers comprising the nucleotide sequence of SEQ ID No 16 and SEQ ID No 17 respectively, yields a DNA fragment of about 183 bp or 183 bp, or with two primers comprising the nucleotide sequence of SEQ ID No 18 and SEQ ID No 19 respectively, yields a DNA fragment of about 151 bp or 151 bp.

Also provided herein is a method for identifying a transgenic soybean plant, or cells, parts, seed or progeny thereof comprising 2 elite events, wherein said plant, cells, seed, or progeny are tolerant to glyphosate, glufosinate and an HPPD inhibitor herbicide (such as isoxaflutole), in biological samples, said method comprising amplifying a DNA fragment of between 100 and 500 bp from a nucleic acid of said first event present in biological samples using a polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of the first elite event specified above, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451, or the 3' flanking region of said first elite event, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, the other primer of said primers recognizing a sequence within the foreign DNA of said first event comprising the nucleotide sequence of the complement of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, and comprising amplifying a DNA fragment of between 50 and 1000 bp, or between 100 and 500 hp from a nucleic acid of said second event present in biological samples using a polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of the second elite event specified above, said 5' flanking region comprising the nucleotide sequence of nucleotides 1 to 209 of SEQ ID No 12, or the 3' flanking region of said second elite event, said 3' flanking region comprising the nucleotide sequence of the complement of nucleotides 569 to 1000 of SEQ ID No 13, the other primer of said primers recognizing a sequence within the foreign DNA of said second event comprising the nucleotide sequence of the complement of SEQ ID No 12 from nucleotide 210 to nucleotide 720 or the nucleotide sequence of SEQ ID No 13 from nucleotide 1 to nucleotide 568.

Also provided herein is a method for identifying a transgenic soybean plant, or cells, parts, seed or progeny thereof comprising 2 elite events, wherein said plant, cells, seed, or progeny are tolerant to glyphosate, glufosinate and/or an HPPD inhibitor herbicide (such as isoxaflutole), in biological samples, said method comprising amplifying a DNA fragment of between 50 and 1000 or between 100 and 500 bp from a nucleic acid of said first event present in biological samples using a polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of the first elite event specified above, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451, or the 3' flanking region of said first elite event, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, the other primer of said primers recognizing a sequence within the foreign DNA of said first event comprising the nucleotide sequence of the complement of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, and comprising amplifying a DNA fragment of between 50 and 1000 bp or between 100 and 500 bp from a nucleic acid of said second event present in biological samples using a polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of the second elite event specified above, said 5' flanking region comprising the nucleotide sequence of nucleotides 1 to 311 of SEQ ID No 14, or the 3' flanking

region of said second elite event, said 3' flanking region comprising the nucleotide sequence of the complement of nucleotides 508 to 1880 of SEQ ID No 15, the other primer of said primers recognizing a sequence within the foreign DNA of said second event comprising the nucleotide sequence of the complement of SEQ ID No 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No 15 from nucleotide 1 to nucleotide 507.

Also provided herein is a kit for identifying a transgenic soybean plant, or cells, parts, seed or progeny thereof comprising 2 elite events and being tolerant to glyphosate, glufosinate and an HPPD inhibitor herbicide (such as isoxaflutole), in biological samples, said kit comprising one primer recognizing the 5' flanking region of the first elite event specified above, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451, or one primer recognizing the 3' flanking region of said first elite event, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, and one primer recognizing a sequence within the foreign DNA of said first event, said foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, and said kit comprising one primer recognizing the 5' flanking region of the second elite event specified above, said 5' flanking region comprising the nucleotide sequence of nucleotides 1 to 209 of SEQ ID No 12, or one primer recognizing the 3' flanking region of said second elite event, said 3' flanking region comprising the nucleotide sequence of the complement of nucleotides 569 to 1000 of SEQ ID No 13, the other primer of said primers recognizing a sequence within the foreign DNA of said second event comprising the nucleotide sequence of the complement of SEQ ID No 12 from nucleotide 210 to nucleotide 720 or the nucleotide sequence of SEQ ID No 13 from nucleotide 1 to nucleotide 568.

Also provided herein is a kit for identifying a transgenic soybean plant, or cells, parts, seed or progeny thereof comprising 2 elite events and being tolerant to glyphosate, glufosinate and an HPPD inhibitor herbicide (such as isoxaflutole), in biological samples, said kit comprising one primer recognizing the 5' flanking region of the first elite event specified above, said 5' flanking region comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1 to nucleotide 1451, or one primer recognizing the 3' flanking region of said first elite event, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408, and one primer recognizing a sequence within the foreign DNA of said first event, said foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No 3 from nucleotide 1 to nucleotide 240, and said kit comprising one primer recognizing the 5' flanking region of the second elite event specified above, said 5' flanking region comprising the nucleotide sequence of nucleotides 1 to 311 of SEQ ID No 14, or the 3' flanking region of said second elite event, said 3' flanking region comprising the nucleotide sequence of the complement of nucleotides 508 to 1880 of SEQ ID No 15, the other primer of said primers recognizing a sequence within the foreign DNA of said second event comprising the nucleotide sequence of the complement of SEQ ID No 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No 15 from nucleotide 1 to nucleotide 507.

Also provided herein is a soybean plant, plant cell, tissue, or seed, comprising in their genome a nucleic acid molecule comprising a nucleotide sequence with at least 97, 98, or at least 99% or 99.5% sequence identity to the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638, the nucleotide sequence of SEQ ID No. 20 from nucleotide position 2257 to nucleotide position 16601 or their complement, or a nucleotide sequence with at least 97, 98, or at least 99% or 99.5% sequence identity to SEQ ID No. 20 or the complement thereof, and comprising in their genome a nucleic acid molecule comprising a nucleotide sequence with at least 97, 98, or at least 99% or 99.5% sequence identity to the nucleotide sequence of EE-GM1 or EE-GM2 or the complement thereof, reference seed comprising EE-GM1 having been deposited under deposit number NCIMB 41658, and reference seed comprising EE-GM2 having been deposited under deposit number NCIMB 41660. In one embodiment of this invention, the nucleotide sequence of EE-GM1 or EE-GM2 in said plant, plant cell, tissue, or seed, is the DNA sequence (such as the foreign DNA sequence) in SEQ ID No 12 or 13, or the DNA sequence (such as the foreign DNA sequence) in SEQ ID No 14 or 15, or the DNA sequence in the plant genome (such as in the deposited seeds comprising EE-GM1 or EE-GM2 of the invention) comprising SEQ ID No 12 and 13 between the first nucleotide of the sequence of SEQ ID No 12 and the last nucleotide of the sequence of SEQ ID No 13, or the DNA sequence in the plant genome comprising SEQ ID No 14 and 15 between the first nucleotide of the sequence of SEQ ID No 14 and the last nucleotide of the sequence of SEQ ID No 15.

One embodiment of this invention provides a soybean plant, plant cell, tissue, or seed, comprising in their genome a nucleic acid molecule hybridizing to the nucleotide sequence of SEQ ID No 1 or the complement thereof, or hybridizing to the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or the complement thereof, or hybridizing to the nucleotide sequence of SEQ ID No. 20 or the complement thereof, and comprising in their genome a nucleic acid molecule hybridizing to the nucleotide sequence of EE-GM1 or EE-GM2 or the complement thereof, reference seed comprising EE-GM1 having been deposited under deposit number NCIMB 41658, and reference seed comprising EE-GM2 having been deposited under deposit number NCIMB 41660. In one embodiment of this invention, the nucleotide sequence of EE-GM1 or EE-GM2 in said plant, plant cell, tissue, or seed, is the foreign DNA in SEQ ID No 12 or 13, or the foreign DNA in SEQ ID No 14 or 15, or the foreign DNA sequence in the plant genome (such as in the deposited seeds comprising EE-GM1 or EE-GM2 of the invention) comprising SEQ ID No 12 and 13 between the first nucleotide of the sequence of SEQ ID No 12 and the last nucleotide of the sequence of SEQ ID No 13, or the foreign DNA sequence in the plant genome comprising SEQ ID No 14 and 15 between the first nucleotide of the sequence of SEQ ID No 14 and the last nucleotide of the sequence of SEQ ID No 15.

BRIEF DESCRIPTION OF THE DRAWINGS

The following Examples, not intended to limit the invention to specific embodiments described, may be understood in conjunction with the accompanying Figures, incorporated herein by reference, in which:

FIG. **1**: Schematic representation of the relationship between the cited nucleotide sequences and primers for elite event EE-GM3. black bar: foreign DNA; hatched bar: DNA

of plant origin; checkered arrow (a): chimeric HPPD PF W366 encoding gene (see Table 1 for composition of the chimeric gene); hatched arrow (b): chimeric 2mEPSPS encoding gene (see Table 1 for composition of the chimeric gene); black arrows: oligonucleotide primers, the figures under the bars represent nucleotide positions; (c) refers to complement of the indicated nucleotide sequence; Note: the scheme is not drawn to scale.

FIG. **2**: Results obtained by the PCR Identification Protocol developed for EE-GM3. Loading sequence of the gel: Lane 1: Molecular weight marker (100 bp ladder); lanes 2 and 3: DNA samples from soybean plants comprising the transgenic event EE-GM3; lanes 4-7: DNA samples from transgenic soybean plants not comprising elite event EE-GM3, but comprising the same herbicide tolerance genes (other transformation events); lane 8: DNA sample from wt soybean; lane 9: no template DNA control; lane 10: molecular weight marker.

FIG. **3**: Schematic representation of the relationship between the cited nucleotide sequences and primers for elite event EE-GM1. black bar: foreign DNA; hatched bar: DNA of plant origin; horizontally striped arrow (d): chimeric phosphinothricin acetyltransferase encoding gene (see SEQ ID No. 11 for composition of the chimeric gene; black arrows: oligonucleotide primers, the figures under the bars represent nucleotide positions; (c) refers to complement of the indicated nucleotide sequence; Note: the scheme is not drawn to scale.

FIG. **4**: Schematic representation of the relationship between the cited nucleotide sequences and primers for elite event EE-GM2. black bar: foreign DNA; hatched bar: DNA of plant origin; horizontally striped arrow (d): chimeric phosphinothricin acetyltransferase encoding gene (see SEQ ID No. 11 for composition of the chimeric gene; black arrows: oligonucleotide primers, the figures under the bars represent nucleotide positions; (c) refers to complement of the indicated nucleotide sequence; NA: not applicable. Note: the scheme is not drawn to scale.

FIG. **5**: PCR Identification Protocol developed for EE-GM1. Loading sequence of the gel: Lane1: DNA sample from soybean plants comprising the transgenic event EE-GM1; lane 2: DNA sample from a transgenic soybean plant not comprising elite event EE-GM1; lane 3: control DNA samples from wild-type soybean plants; lane 4: no template control; lane 5: molecular weight marker.

FIG. **6**: PCR Identification Protocol developed for EE-GM2. Loading sequence of the gel: Lane 1: DNA sample from soybean plants comprising the transgenic event EE-GM2; lane 2: DNA sample from a transgenic soybean plant not comprising elite event EE-GM2; lane 3: control DNA samples from wild-type soybean plants; lane 4: no template control; lane 5: molecular weight marker.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The incorporation of a recombinant DNA molecule in the plant genome typically results from transformation of a cell or tissue. The particular site of incorporation is usually due to random integration.

The DNA introduced into the plant genome as a result of transformation of a plant cell or tissue with a recombinant DNA or "transforming DNA", and originating from such transforming DNA is hereinafter referred to as "foreign DNA" comprising one or more "transgenes". The transgenes of EE-GM3 are the glyphosate and HPPD inhibitor herbicide tolerance genes. "Plant DNA" in the context of the present invention will refer to DNA originating from the plant which is transformed. Plant DNA will usually be found in the same genetic locus in the corresponding wild-type plant. The foreign DNA can be characterized by the location and the configuration at the site of incorporation of the recombinant DNA molecule in the plant genome. The site in the plant genome where a recombinant DNA has been inserted is also referred to as the "insertion site" or "target site". Insertion of the recombinant DNA into the region of the plant genome referred to as "pre-insertion plant DNA" can be associated with a deletion of plant DNA, referred to as "target site deletion". A "flanking region" or "flanking sequence" as used herein refers to a sequence of at least 20 bp, preferably at least 50 bp, and up to 5000 bp of DNA different from the introduced DNA, preferably DNA from the plant genome which is located either immediately upstream of and contiguous with or immediately downstream of and contiguous with the foreign DNA. Transformation procedures leading to random integration of the foreign DNA will result in transformants with different flanking regions, which are characteristic and unique for each transformant. When the recombinant DNA is introduced into a plant through traditional crossing, its insertion site in the plant genome, or its flanking regions will generally not be changed.

An "isolated nucleic acid (sequence)" or "isolated DNA (sequence)", as used herein, refers to a nucleic acid or DNA (sequence) which is no longer in the natural environment it was isolated from, e.g., the nucleic acid sequence in another bacterial host or in a plant genome, or a nucleic acid or DNA fused to DNA or nucleic acid from another origin, such as when contained in a chimeric gene under the control of a plant-expressible promoter.

An event is defined as a (artificial) genetic locus that, as a result of genetic engineering, carries a foreign DNA or transgene comprising at least one copy of a gene of interest or of the genes of interest. The typical allelic states of an event are the presence or absence of the foreign DNA. An event is characterized phenotypically by the expression of the transgene. At the genetic level, an event is part of the genetic make-up of a plant. At the molecular level, an event can be characterized by the restriction map (e.g., as determined by Southern blotting), by the upstream and/or downstream flanking sequences of the transgene, the location of molecular markers and/or the molecular configuration of the transgene. Usually transformation of a plant with a transforming DNA comprising at least one gene of interest leads to a population of transformants comprising a multitude of separate events, each of which is unique. An event is characterized by the foreign DNA and at least one of the flanking sequences.

An elite event, as used herein, is an event which is selected from a group of events, obtained by transformation with the same transforming DNA, based on the expression and stability of the transgene(s) and its compatibility with optimal agronomic characteristics of the plant comprising it. Thus the criteria for elite event selection are one or more, preferably two or more, advantageously all of the following:

a) that the presence of the foreign DNA does not compromise other desired characteristics of the plant, such as those relating to agronomic performance or commercial value;
b) that the event is characterized by a well defined molecular configuration which is stably inherited and for which appropriate tools for identity control can be developed;

c) that the gene(s) of interest show(s) a correct, appropriate and stable spatial and temporal phenotypic expression, both in heterozygous (or hemizygous) and homozygous condition of the event, at a commercially acceptable level in a range of environmental conditions in which the plants carrying the event are likely to be exposed in normal agronomic use.

It is preferred that the foreign DNA is associated with a position in the plant genome that allows easy introgression into desired commercial genetic backgrounds.

The status of an event as an elite event is confirmed by introgression of the elite event in different relevant genetic backgrounds and observing compliance with one, two or all of the criteria e.g. a), b) and c) above.

An "elite event" thus refers to a genetic locus comprising a foreign DNA, which meets the above-described criteria. A plant, plant material or progeny such as seeds can comprise one or more elite events in its genome.

The tools developed to identify an elite event or the plant or plant material comprising an elite event, or products which comprise plant material comprising the elite event, are based on the specific genomic characteristics of the elite event, such as, a specific restriction map of the genomic region comprising the foreign DNA, molecular markers or the sequence of the flanking region(s) of the foreign DNA.

Once one or both of the flanking regions of the foreign DNA have been sequenced, primers and probes can be developed which specifically recognize this (these) sequence(s) in the nucleic acid (DNA or RNA) of a sample by way of a molecular biological technique. For instance a PCR method can be developed to identify the elite event in biological samples (such as samples of plants, plant material or products comprising plant material). Such a PCR is based on at least two specific "primers", one recognizing a sequence within the 5' or 3' flanking region of the elite event and the other recognizing a sequence within the foreign DNA. The primers preferably have a sequence of between 15 and 35 nucleotides which under optimized PCR conditions "specifically recognize" a sequence within the 5' or 3' flanking region of the elite event and the foreign DNA of the elite event respectively, so that a specific fragment ("integration fragment" or discriminating amplicon) is amplified from a nucleic acid sample comprising the elite event. This means that only the targeted integration fragment, and no other sequence in the plant genome or foreign DNA, is amplified under optimized PCR conditions.

PCR primers suitable for identification of EE-GM3 may be the following:

oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the plant DNA in the 5' flanking sequence (SEQ ID No 2 from nucleotide 1 to nucleotide 1451) at their 3' end (primers recognizing 5' flanking sequences); or

oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the plant DNA in the 3' flanking sequence (complement of SEQ ID No 3 from nucleotide 241 to nucleotide 1408) at their 3' end (primers recognizing 3' flanking sequences); or

oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the inserted DNA sequences (complement of SEQ ID No 2 from nucleotide 1452 to nucleotide 1843) at their 3' end (primers recognizing foreign DNA); or

oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the inserted DNA sequences (SEQ ID No 3 from nucleotide 1 to nucleotide 240); or

suitable oligonucleotides ranging in length from 17 nt to about 200 nt, comprising a nucleotide sequence of at least 17 consecutive nucleotides, preferably 20 consecutive nucleotides, selected from the nucleotide sequence of the inserted DNA fragment or its complement (SEQ ID No 1 or SEQ ID No 20 from nucleotide position 1452 to 16638).

The printers may of course be longer than the mentioned 17 consecutive nucleotides, and may, e.g., be 20, 21, 30, 35, 50, 75, 100, 150, 200 nt long or even longer. The primers may entirely consist of nucleotide sequence selected from the mentioned nucleotide sequences of flanking sequences and foreign DNA sequences. However, the nucleotide sequence of the primers at their 5' end (i.e. outside of the 3'-located 17 consecutive nucleotides) is less critical. Thus, the 5' sequence of the primers may comprise or consist of a nucleotide sequence selected from the flanking sequences or foreign DNA, as appropriate, but may contain several (e.g., 1, 2, 5, or 10) mismatches. The 5' sequence of the primers may even entirely be a nucleotide sequence unrelated to the flanking sequences or foreign DNA, such as, e.g., a nucleotide sequence representing one or more restriction enzyme recognition sites. Such unrelated sequences or flanking DNA sequences with mismatches should preferably be not longer than 100, more preferably not longer than 50 or even 25 nucleotides.

Moreover, suitable primers may comprise, consist or consist essentially of a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences (located at nucleotides 1451-1452 in SEQ ID No 2 and nucleotides 240-241 in SEQ ID No 3 for EE-GM3) provided the mentioned 3'-located 17 consecutive nucleotides are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID No 2 or 3.

It will also be immediately clear to the skilled artisan that properly selected PCR primer pairs should also not comprise sequences complementary to each other.

For the purpose of the invention, the "complement of a nucleotide sequence represented in SEQ ID No: X" is the nucleotide sequence which can be derived from the represented nucleotide sequence by replacing the nucleotides with their complementary nucleotide according to Chargaff's rules (A⇔T; G⇔C) and reading the sequence in the 5' to 3' direction, i.e., in opposite direction of the represented nucleotide sequence.

Examples of suitable primers for EE-GM3 are the oligonucleotide sequences of SEQ ID No 5 (3' flanking sequence recognizing primer), SEQ ID No 4 (foreign DNA recognizing primer for use with a 3' flanking sequence recognizing primers), or SEQ ID No 7 (foreign DNA recognizing primer for use with a 3' flanking sequence recognizing primers).

Other examples of suitable oligonucleotide primers for EE-GM3 comprise at their 3' end the following sequences or consist (essentially) of such sequences:

a. 5' flanking sequence recognizing primers:

the nucleotide sequence of SEQ ID No 2 from nucleotide 264 to nucleotide 283

the nucleotide sequence of SEQ ID No 2 from nucleotide 266 to nucleotide 285
the nucleotide sequence of SEQ ID No 2 from nucleotide 1240 to nucleotide 1259
the nucleotide sequence of SEQ ID No 2 from nucleotide 265 to nucleotide 285
the nucleotide sequence of SEQ ID No 2 from nucleotide 265 to nucleotide 283
the nucleotide sequence of SEQ ID No 2 from nucleotide 1239 to nucleotide 1259
the nucleotide sequence of SEQ ID No 2 from nucleotide 1241 to nucleotide 1259
the nucleotide sequence of SEQ ID No 2 from nucleotide 1244 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1248 to nucleotide 1267
the nucleotide sequence of SEQ ID No 2 from nucleotide 1250 to nucleotide 1269
the nucleotide sequence of SEQ ID No 2 from nucleotide 262 to nucleotide 279
the nucleotide sequence of SEQ ID No 2 from nucleotide 263 to nucleotide 279
the nucleotide sequence of SEQ ID No 2 from nucleotide 264 to nucleotide 285
the nucleotide sequence of SEQ ID No 2 from nucleotide 266 to nucleotide 283
the nucleotide sequence of SEQ ID No 2 from nucleotide 1238 to nucleotide 1259
the nucleotide sequence of SEQ ID No 2 from nucleotide 1242 to nucleotide 1259
the nucleotide sequence of SEQ ID No 2 from nucleotide 1243 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1245 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1247 to nucleotide 1267
the nucleotide sequence of SEQ ID No 2 from nucleotide 1249 to nucleotide 1269
the nucleotide sequence of SEQ ID No 2 from nucleotide 1249 to nucleotide 1267
the nucleotide sequence of SEQ ID No 2 from nucleotide 263 to nucleotide 285
the nucleotide sequence of SEQ ID No 2 from nucleotide 267 to nucleotide 283
the nucleotide sequence of SEQ ID No 2 from nucleotide 1242 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1243 to nucleotide 1259
the nucleotide sequence of SEQ ID No 2 from nucleotide 1246 to nucleotide 1267
the nucleotide sequence of SEQ ID No 2 from nucleotide 1246 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1248 to nucleotide 1269
the nucleotide sequence of SEQ ID No 2 from nucleotide 1250 to nucleotide 1271
the nucleotide sequence of SEQ ID No 2 from nucleotide 1250 to nucleotide 1267
the nucleotide sequence of SEQ ID No 2 from nucleotide 1241 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1245 to nucleotide 1267
the nucleotide sequence of SEQ ID No 2 from nucleotide 1247 to nucleotide 1269
the nucleotide sequence of SEQ ID No 2 from nucleotide 1247 to nucleotide 1263
the nucleotide sequence of SEQ ID No 2 from nucleotide 1249 to nucleotide 1271
the nucleotide sequence of SEQ ID No 2 from nucleotide 1242 to nucleotide 1261
the nucleotide sequence of SEQ ID No 2 from nucleotide 1241 to nucleotide 1261
the nucleotide sequence of SEQ ID No 2 from nucleotide 1243 to nucleotide 1261
the nucleotide sequence of SEQ ID No 2 from nucleotide 1240 to nucleotide 1261
the nucleotide sequence of SEQ ID No 2 from nucleotide 1244 to nucleotide 1261
the nucleotide sequence of SEQ ID No 2 from nucleotide 1239 to nucleotide 1261
the nucleotide sequence of SEQ ID No 2 from nucleotide 1245 to nucleotide 1261

b. foreign DNA sequence recognizing primers for use with 5' flanking sequence recognizing primers:

the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1751
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1735 to nucleotide 1754
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1750
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1750
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1752
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1749
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1749
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1751
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1753
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1748
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1748
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1735 to nucleotide 1751
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1752
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1732 to nucleotide 1754
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1747
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1731 to nucleotide 1753
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1746
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1745
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1747
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1744
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1748
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1727 to nucleotide 1749
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1745
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1744

the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1746
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1747
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1726 to nucleotide 1748
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1724 to nucleotide 1744
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1724 to nucleotide 1745
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1724 to nucleotide 1746
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1461 to nucleotide 1478
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1686
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1486
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1508 to nucleotide 1527
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1686
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1687
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1689
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1704
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1705
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1709
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1486
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1497
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1498
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1507
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1508
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1688
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1690
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1691
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1706
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1707
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1708
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1487
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1499
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1505
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1506
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1507
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1508
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1666 to nucleotide 1686
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1687
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1688
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1689
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1707
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1709
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1710
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1500
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1509
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1689
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1690
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1691
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1705
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1706
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1710
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1488
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1507
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1510
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1512
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1513
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1514
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1692
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1694
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1695
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1696
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1703
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1704
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1711
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1488
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1506
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1511
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1690
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1697

the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1709
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1487
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1508
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1511
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1491 to nucleotide 1512
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1666 to nucleotide 1687
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1688
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1672 to nucleotide 1692
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1693
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1707
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1490
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1491
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1501
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1509
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1495 to nucleotide 1515
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1691
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1673 to nucleotide 1694
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1698
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1489
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1667 to nucleotide 1689
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1708
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1688 to nucleotide 1710
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1711
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1492
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1510
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1666 to nucleotide 1688
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1687 to nucleotide 1709
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1712
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1488
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1490
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1509
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1489 to nucleotide 1511
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1699
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1493
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1472 to nucleotide 1494
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1502
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1670 to nucleotide 1692
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1469 to nucleotide 1491
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1488 to nucleotide 1510
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1712
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1713
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1692 to nucleotide 1714
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1467 to nucleotide 1489
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1678 to nucleotide 1700
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1481 to nucleotide 1503
the complement of the nucleotide sequence of SEQ ID No 2 from nucleotide 1691 to nucleotide 1713

c. 3' flanking sequence recognizing primers:
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 847
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 849
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 846
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 848
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 848
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 850
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 845
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 847
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 849
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 851
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 844
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 846
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 828 to nucleotide 850
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 830 to nucleotide 852
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 992 to nucleotide 1009
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 731 to nucleotide 752
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 795
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 731 to nucleotide 753
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 794
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 796

the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 793
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 797
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 792
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 776 to nucleotide 798
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 752
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 753
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 754
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 733 to nucleotide 755
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 838 to nucleotide 854
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 246 to nucleotide 263
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 838 to nucleotide 855
the complement of the nucleotide sequence of SEQ ID No 3 from nucleotide 245 to nucleotide 264

d. foreign DNA sequence recognizing primers for use with 3' flanking sequence recognizing primers:
the nucleotide sequence of SEQ ID No 3 from nucleotide 173 to nucleotide 192
the nucleotide sequence of SEQ ID No 3 from nucleotide 22 to nucleotide 41
the nucleotide sequence of SEQ ID No 3 from nucleotide 172 to nucleotide 192
the nucleotide sequence of SEQ ID No 3 from nucleotide 174 to nucleotide 192
the nucleotide sequence of SEQ ID No 3 from nucleotide 191 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 171 to nucleotide 192
the nucleotide sequence of SEQ ID No 3 from nucleotide 175 to nucleotide 192
the nucleotide sequence of SEQ ID No 3 from nucleotide 190 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 192 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 176 to nucleotide 192
the nucleotide sequence of SEQ ID No 3 from nucleotide 189 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 193 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 188 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 194 to nucleotide 210
the nucleotide sequence of SEQ ID No 3 from nucleotide 199 to nucleotide 218
the nucleotide sequence of SEQ ID No 3 from nucleotide 200 to nucleotide 218
the nucleotide sequence of SEQ ID No 3 from nucleotide 197 to nucleotide 218
the nucleotide sequence of SEQ ID No 3 from nucleotide 201 to nucleotide 218
the nucleotide sequence of SEQ ID No 3 from nucleotide 201 to nucleotide 220
the nucleotide sequence of SEQ ID No 3 from nucleotide 200 to nucleotide 220
the nucleotide sequence of SEQ ID No 3 from nucleotide 199 to nucleotide 220
the nucleotide sequence of SEQ ID No 3 from nucleotide 200 to nucleotide 221
the nucleotide sequence of SEQ ID No 3 from nucleotide 199 to nucleotide 221
the nucleotide sequence of SEQ ID No 3 from nucleotide 150 to nucleotide 172

PCR primers suitable for the identification of EE-GM1 have been described in WO2006/108674, particularly on page 8, line 4 to page 26, line 7 (herein incorporated by reference).

PCR primers suitable for the identification of EE-GM2 have been described in WO2006/108675, particularly on pages 8, line 4 to page 33, line 4 (herein incorporated by reference).

As used herein, "the nucleotide sequence of SEQ ID No. Z from position X to position Y" indicates the nucleotide sequence including both nucleotide endpoints.

Preferably, the amplified fragment has a length of between 50 and 500 nucleotides, such as a length between 100 and 350 nucleotides. The specific primers may have a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event and the foreign DNA of the elite event, respectively, provided the mismatches still allow specific identification of the elite event with these primers under optimized PCR conditions. The range of allowable mismatches however, can easily be determined experimentally and are known to a person skilled in the art.

Detection of integration fragments can occur in various ways, e.g., via size estimation after gel analysis. The integration fragments may also be directly sequenced. Other sequence specific methods for detection of amplified DNA fragments are also known in the art.

As the sequence of the primers and their relative location in the genome are unique for the elite event, amplification of the integration fragment will occur only in biological samples comprising (the nucleic acid of) the elite event. Preferably when performing a PCR to identify the presence of elite events EE-GM3 and EE-GM1 or elite events EE-GM3 and EE-GM2 in unknown samples, a control is included of a set of primers with which a fragment within a "housekeeping gene" of the plant species of the event can be amplified. Housekeeping genes are genes that are expressed in most cell types and which are concerned with basic metabolic activities common to all cells. Preferably, the fragment amplified from the housekeeping gene is a fragment which is larger than the amplified integration fragment. Depending on the samples to be analyzed, other controls can be included.

Standard PCR protocols are described in the art, such as in 'PCR Applications Manual" (Roche Molecular Biochemicals, 2nd Edition, 1999) and other references. The optimal conditions for the PCR, including the sequence of the specific primers, are specified in a "PCR (or Polymerase Chain Reaction) Identification Protocol" for each elite event. It is however understood that a number of parameters in the PCR Identification Protocol may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA may require adjustment of, for instance, the amount of primers, polymerase and annealing conditions used. Similarly, the selection of other primers may dictate other optimal conditions for the PCR Identification Protocol. These adjustments will however be appar-

ent to a person skilled in the art, and are furthermore detailed in current PCR application manuals such as the one cited above.

Alternatively, specific primers can be used to amplify integration fragments that can be used as "specific probes" for identifying EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2 in biological samples. Contacting nucleic acid of a biological sample, with the probes, under conditions which allow hybridization of the probes with their corresponding fragments in the sample nucleic acid, results in the formation of nucleic acid/probe hybrids. The formation of these hybrids can be detected (e.g. via labeling of the nucleic acid or probe), whereby the formation of these hybrids indicates the presence of EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2. Such identification methods based on hybridization with a specific probe (either on a solid phase carrier or in solution) have been described in the art. The specific probe is preferably a sequence which, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region of the elite event and preferably also comprising part of the foreign DNA contiguous therewith (hereinafter referred to as "specific region"). Preferably, the specific probe comprises a sequence of between 50 and 500 bp, preferably of 100 to 350 bp which is at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, most preferably between 95% and 100% identical (or complementary) to the nucleotide sequence of a specific region. Preferably, the specific probe will comprise a sequence of about 15 to about 100 contiguous nucleotides identical (or complementary) to a specific region of the elite event.

Furthermore, detection methods specific for elite events EE-GM3 and EE-GM1 or for elite events EE-GM3 and EE-GM2 which differ from PCR based amplification methods can also be developed using the elite event specific sequence information provided herein. Such alternative detection methods include linear signal amplification detection methods based on invasive cleavage of particular nucleic acid structures, also known as Invader™ technology, (as described e.g. in U.S. Pat. No. 5,985,557 "Invasive Cleavage of Nucleic Acids", U.S. Pat. No. 6,001,567 "Detection of Nucleic Acid sequences by Invader Directed Cleavage, incorporated herein by reference). For EE-GM3 detection by this method, the target sequence may hybridized with a labeled first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1452 to nucleotide 1469 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 223 to nucleotide 240 or its complement and is further hybridized with a second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 2 from nucleotide 1434 to nucleotide 1451 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 3 from nucleotide 241 to nucleotide 258 or its complement, wherein the first and second oligonucleotide overlap by at least one nucleotide. The duplex or triplex structure which is produced by this hybridization allows selective probe cleavage with an enzyme (Cleavase®) leaving the target sequence intact. The cleaved labeled probe is subsequently detected, potentially via an intermediate step resulting in further signal amplification. For EE-GM1 detection by this method, the target sequence may hybridized with a labeled first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 12 from nucleotide 210 to nucleotide 227 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 13 from nucleotide 561 to nucleotide 568 or its complement and is further hybridized with a second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 12 from nucleotide 192 to nucleotide 209 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 13 from nucleotide 569 to nucleotide 586 or its complement, wherein the first and second oligonucleotide overlap by at least one nucleotide. The duplex or triplex structure which is produced by this hybridization allows selective probe cleavage with an enzyme (Cleavase®) leaving the target sequence intact. The cleaved labeled probe is subsequently detected, potentially via an intermediate step resulting in further signal amplification. For EE-GM2 detection by this method, the target sequence may hybridized with a labeled first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 14 from nucleotide 312 to nucleotide 329 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 15 from nucleotide 490 to nucleotide 507 or its complement and is further hybridized with a second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No 14 from nucleotide 294 to nucleotide 311 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No 15 from nucleotide 508 to nucleotide 525 or its complement, wherein the first and second oligonucleotide overlap by at least one nucleotide. The duplex or triplex structure which is produced by this hybridization allows selective probe cleavage with an enzyme (Cleavase®) leaving the target sequence intact. The cleaved labeled probe is subsequently detected, potentially via an intermediate step resulting in further signal amplification.

A "kit" as used herein refers to a set of reagents for the purpose of performing the method of the invention, more particularly, the identification of the elite event EE-GM3 in biological samples or the determination of the zygosity status of EE-GM3 containing plant material. More particularly, a preferred embodiment of the kit of the invention comprises at least one or two specific primers, as described above for identification of the elite event, or three specific primers for the determination of the zygosity status. Optionally, the kit can further comprise any other reagent described herein in the PCR Identification Protocol. Alternatively, according to another embodiment of this invention, the kit can comprise a specific probe, as described above, which specifically hybridizes with nucleic acid of biological samples to identify the presence of EE-GM3 therein. Optionally, the kit can further comprise any other reagent (such as but not limited to hybridizing buffer, label) for identification of EE-GM3 in biological samples, using the specific probe.

The kit of the invention can be used, and its components can be specifically adjusted, for purposes of quality control (e.g., purity of seed lots), detection of the presence or absence of the elite event in plant material or material comprising or derived from plant material, such as but not limited to food or feed products.

As used herein, "sequence identity" with regard to nucleotide sequences (DNA or RNA), refers to the number of positions with identical nucleotides divided by the number of nucleotides in the shorter of the two sequences. The alignment of the two nucleotide sequences is performed by the Wilbur and Lipmann algorithm (Wilbur and Lipmann, 1983, Proc. Nat. Acad. Sci. USA 80:726) using a window-size of 20 nucleotides, a word length of 4 nucleotides, and a gap penalty of 4. Computer-assisted analysis and interpre-

tation of sequence data, including sequence alignment as described above, can, e.g., be conveniently performed using the sequence analysis software package of the Genetics Computer Group (GCG, University of Wisconsin Biotechnology Center). Sequences are indicated as "essentially similar" when such sequences have a sequence identity of at least about 75%, particularly at least about 80%, more particularly at least about 85%, quite particularly at least about 90%, especially at least about 95%, more especially at least about 98%, or at least about 99%. It is clear that when RNA sequences are said to be essentially similar or have a certain degree of sequence identity with DNA sequences, thymidine (T) in the DNA sequence is considered equal to uracil (U) in the RNA sequence. Also, it is clear that small differences or mutations may appear in DNA sequences over time and that some mismatches can be allowed for the event-specific primers or probes of the invention, so any DNA sequence indicated herein in any embodiment of this invention for any 3' or 5' flanking DNA or for any insert or foreign DNA or any primer or probe of this invention, also includes sequences essentially similar to the sequences provided herein, such as sequences hybridizing to or with at least 90%, 95%, 96%, 97%, 98%, or at least 99% sequence identity to the sequence given for any 3' or 5' flanking DNA, for any primer or probe or for any insert or foreign DNA of this invention.

The term "primer" as used herein encompasses any nucleic acid that is capable of priming the synthesis of a nascent nucleic acid in a template-dependent process, such as PCR. Typically, primers are oligonucleotides from 10 to 30 nucleotides, but longer sequences can be employed. Primers may be provided in double-stranded form, though the single-stranded form is preferred. Probes can be used as primers, but are designed to bind to the target DNA or RNA and need not be used in an amplification process.

The term "recognizing" as used herein when referring to specific primers, refers to the fact that the specific primers specifically hybridize to a nucleic acid sequence in the elite event under the conditions set forth in the method (such as the conditions of the PCR Identification Protocol), whereby the specificity is determined by the presence of positive and negative controls.

The term "hybridizing" as used herein when referring to specific probes, refers to the fact that the probe binds to a specific region in the nucleic acid sequence of the elite event under standard stringency conditions. Standard stringency conditions as used herein refers to the conditions for hybridization described herein or to the conventional hybridizing conditions as described by Sambrook et al., 1989 (Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY) which for instance can comprise the following steps: 1) immobilizing plant genomic DNA fragments on a filter, 2) prehybridizing the filter for 1 to 2 hours at 42° C. in 50% formamide, 5×SSPE, 2×Denhardt's reagent and 0.1% SDS, or for 1 to 2 hours at 68° C. in 6×SSC, 2×Denhardt's reagent and 0.1% SDS, 3) adding the hybridization probe which has been labeled, 4) incubating for 16 to 24 hours, 5) washing the filter for 20 min. at room temperature in 1×SSC, 0.1% SDS, 6) washing the filter three times for 20 min. each at 68° C. in 0.2×SSC, 0.1% SDS, and 7) exposing the filter for 24 to 48 hours to X-ray film at −70° C. with an intensifying screen.

As used in herein, a biological sample is a sample of a plant, plant material or products comprising plant material. The term "plant" is intended to encompass soybean (*Glycine max*) plant tissues, at any stage of maturity, as well as any cells, tissues, or organs taken from or derived from any such plant, including without limitation, any seeds, leaves, stems, flowers, roots, single cells, gametes, cell cultures, tissue cultures or protoplasts. "Plant material", as used herein refers to material which is obtained or derived from a plant. Products comprising plant material relate to food, feed or other products which are produced using plant material or can be contaminated by plant material. It is understood that, in the context of the present invention, such biological samples are tested for the presence of nucleic acids specific for EE-GM3, EE-GM1 and EE-GM2 implying the presence of nucleic acids in the samples. Thus the methods referred to herein for identifying elite event EE-GM3 and EE-GM2 or EE-GM3 and EE-GM1 in biological samples, relate to the identification in biological samples of nucleic acids which comprise the elite event.

As used herein "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps, reagents or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid or protein. A chimeric gene comprising a DNA sequence which is functionally or structurally defined, may comprise additional DNA sequences, such as promoter and transcript termination sequences.

The present invention also relates to the development of a stack of elite event EE-GM3 and elite event EE-GM1 or a stack of elite event EE-GM3 and elite event EE-GM2 in soybean, to the plants comprising a stack of these events, the progeny obtained from these plants and to the plant cells, or plant material derived from plants comprising these stacks. Plants comprising elite event EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2 can be obtained as described in example 1. Stacks are obtained by crossing plants comprising single events using conventional breeding methods and identification of progeny thereof comprising two different events.

Soybean plants or plant material comprising EE-GM3 and EE-GM1 can be identified according to the PCR Identification Protocol described for EE-GM3 and EE-GM1 in Example 2. Briefly, soybean genomic DNA present in the biological sample is amplified by PCR using a primer which specifically recognizes a sequence within the 5' or 3' flanking sequence of EE-GM3 such as the primer with the sequence of SEQ ID NO: 5, and a primer which recognizes a sequence in the foreign DNA, such as the primer with the sequence of SEQ ID NO: 4 and further using a primer which specifically recognizes a sequence within the 5' or 3' flanking sequence of EE-GM1 such as the primer with the sequence of SEQ ID NO: 16, and a primer which recognizes a sequence in the foreign DNA, such as the primer with the sequence of SEQ ID NO: 17.

Soybean plants or plant material comprising EE-GM3 and EE-GM2 can be identified according to the PCR Identification Protocol described for EE-GM3 and EE-GM2 in Example 2. Briefly, soybean genomic DNA present in the biological sample is amplified by PCR using a primer which specifically recognizes a sequence within the 5' or 3' flanking sequence of EE-GM3 such as the primer with the sequence of SEQ ID NO: 5, and a primer which recognizes a sequence in the foreign DNA, such as the primer with the sequence of SEQ ID NO: 4 and further using a primer which specifically recognizes a sequence within the 5' or 3' flanking sequence of EE-GM2 such as the primer with the sequence of SEQ ID

NO: 18, and a primer which recognizes a sequence in the foreign DNA, such as the primer with the sequence of SEQ ID NO: 19.

DNA primers which amplify part of an endogenous soybean sequence are used as positive control for the PCR amplification. If upon PCR amplification, the material yields the fragments of the expected sizes, the material contains plant material from a soybean plant harboring elite event EE-GM3 and EE-GM1 or from a soybean plant harboring elite event EE-GM3 and EE-GM2.

Plants harboring EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2 are characterized by their glyphosate tolerance, as well as by their tolerance to HPPD inhibitors such as isoxaflutol and further by their tolerance to glufosinate. Plants harboring the event stacks are also characterized by having agronomical characteristics that are comparable to commercially available varieties of soybean, in the absence of herbicide application. It has been observed that the presence of foreign DNA in the insertion regions of the soybean plant genome described herein, confers particularly interesting phenotypic and molecular characteristics to the plants comprising this event.

One embodiment of this invention provides a combination or elite events EE-GM3 and EE-GM1 and/or EE-GM2 in soybean plants, obtainable by insertion of transgenes at specific locations in the soybean genome, which elite events confers tolerance to glyphosate, glufosinate and an HPPD inhibitor herbicide such as isoxaflutole on such soybean plants, and wherein such elite events do not cause any effect on the agronomic performance of such soybeans negatively affecting the yield of such soybean plants, compared to isogenic lines (as used herein, "isogenic lines" or "near-isogenic lines" are soybean lines of the same genetic background but lacking the transgenes, such as plants of the same genetic background as the plant used for transformation, or segregating sister lines having lost the transgenes). Particularly, the current invention provides a combination of elite events EE-GM3 and EE-GM1 and/or EE-GM2 in soybean plants, wherein the insertion or presence of said elite event in the genome of such soybean plants does not cause an increased susceptibility to disease, does not cause a yield drag, or does not cause increased lodging, in such soybean plants, as compared to isogenic lines. Hence, the current invention provides a combination of elite events in soybean plants, designated as EE-GM3 and EE-GM1 and/or EE-GM2, which results in soybean plants that can tolerate the application of glyphosate, glufosinate and an HPPD inhibitor herbicide (either simultaneously or separately) without negatively affecting the yield of said soybean plants compared to isogenic lines, which soybean plants have no statistically significant difference in their disease susceptibility, or lodging, compared to isogenic soybean plants. These characteristics make the current combination of elite events very interesting to control glyphosate-resistant weeds in soybean fields, and can also be used in approaches to prevent or delay further glyphosate resistance development in soybean fields (e.g., by application of glyphosate and isoxaflutole and/or glufosinate, or by application of isoxaflutole and glyphosate and/or glufosinate, or by application of glufosinate and glyphosate and/or isoxafulote, securing at least 2 or even 3 different modes of actions of herbicides applied on a soybean field).

Provided herein is also a soybean plant or part thereof comprising event EE-GM3 and elite event EE-GM1 or EE-GM2, wherein representative soybean seed comprising event EE-GM3 has been deposited under NCIMB accession number 41659, representative soybean seed comprising elite event EE-GM1 has been deposited at the NCIMB under accession number NCIMB 41658, representative seed comprising elite event EE-GM2 has been deposited at the NCIMB under accession number NCIMB 41660, representative soybean seed comprising event EE-GM3 and EE-GM1 has been deposited at the ATCC under accession number PTA-11041, and representative soybean seed comprising event EE-GM3 and EE-GM2 has been deposited at the ATCC under accession number PTA-11042.

Soybean plants or parts thereof comprising EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2 may be obtained by combining the respective elite events as can be found in the respective deposited seeds through any means available in the art, including by crossing plants from the deposited seeds, collecting the progeny thereof, and identifying those progeny plants comprising the appropriate combination of elite events. Further provided herein are seeds of such plants, comprising such events, as well as a soybean product produced from such seeds, wherein said soybean product comprises event EE-GM3 and EE-GM1 or EE-GM2. Such soybean product can be or can comprise meal, ground seeds, flour, flakes, etc. Particularly, such soybean product comprises a nucleic acid that produces amplicons diagnostic for event EE-GM3 and elite event EE-GM1 or EE-GM2, such amplicons comprising SEQ ID No. 2 or 3, SEQ ID No. 14 or 15 and/or SEQ ID No. 12 or 13. Also provided herein is a method for producing a soybean product, comprising obtaining soybean seed comprising event EE-GM3 and elite event EE-GM1 or EE-GM2, and producing such soybean product therefrom.

Also provided herein is a soybean plant, which is progeny of any of the above soybean plants, and which comprises event EE-GM3 and EE-GM1 or EE-GM2.

Further provided herein is a method for producing a soybean plant tolerant to glyphosate and/or glufosinate and/or isoxaflutole herbicides, comprising introducing into the genome of such plant event EE-GM3 and event EE-GM1 or EE-GM2, particularly by crossing a first soybean plant containing event EE-GM3 with a soybean plant comprising EE-GM1 and/or EE-GM2, and selecting a progeny plant tolerant to glyphosate and/or glufosinate and/or isoxaflutole.

Also provided herein is a glyphosate and glufosinate and/or isoxaflutole tolerant plant, particularly without yield drag, and with acceptable agronomical characteristics, comprising a 2mEPSPS, HPPD and PAT protein, and capable of producing an amplicon diagnostic for events EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2.

Further provided herein is a method for controlling weeds in a field of soybean plants comprising events EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2, or a field to be planted with such soybean plants, comprising treating the field with an effective amount of an isoxaflutole-based, glyphosate-based and/or glufosinate-based herbicide, wherein such plants are tolerant to such herbicide(s).

Further provided herein is a soybean plant, cell, tissue or seed, comprising EE-GM3 and EE-GM1, characterized by comprising in the genome of its cells a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 2 from nucleotide 1431 to 1472 and a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 3 from nucleotide 220 to 261, or the complement of said sequences, and also a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 12 from nucleotide 199 to 220 and a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 13 from nucleotide 558 to 579, or the complement of said sequences.

Also provided herein is a soybean plant, cell, tissue or seed, comprising EE-GM3 and EE-GM2, characterized by comprising in the genome of its cells a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 2 from nucleotide 1431 to 1472 and a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 3 from nucleotide 220 to 261, or the complement of said sequences, and also a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 14 from nucleotide 301 to 322 and a nucleic acid sequence with at least 80%, 90%, 95% or 100% sequence identity to SEQ ID No. 15 from nucleotide 497 to 518, or the complement of said sequences.

The term "isoxaflutole", as used herein, refers to the herbicide isoxaflutole [i.e. (5-cyclopropyl-4-isoxazolyl)[2-(methylsulfonyl)-4-(trifluoromethyl)phenyl]methanone], the active metabolite thereof, diketonitrile, and any mixtures or solutions comprising said compounds. HPPD inhibiting herbicides useful for application on the plants comprising the events of this invention are the diketonitriles, e.g. 2-cyano-3-cyclopropyl-1-(2-methylsulphonyl-4-trifluoromethylphenyl)-propane-1,3-dione and 2-cyano-1-[4-(methylsulphonyl)-2-trifluoromethylphenyl]-3-(1-methylcyclopropyl)propane-1,3-fione; other isoxazoles; and the pyrazolinates, e.g. topramezonc [i.e.[3-(4,5-dihydro-3-isoxazolyl)-2-methyl-4-(methylsulfonyl)phenyl](5-hydroxy-1-methyl-1H-pyrazol-4-yl)methanone], and pyrasulfotole [(5-hydroxy-1,3-dimethylpyrazol-4-yl(2-mesyl-4-trifluaromethylphenyl)methanone]; or pyrazofen [2-[4-(2,4-dichlorobenzoyl)-1,3-dimethylpyrazol-5-yloxy] acetophenone].

In one embodiment of this invention, a field to be planted with soybean plants containing the EE-GM3 event, can be treated with an HPPD inhibitor herbicide, such as isoxaflutole ('IFT'), before the soybean plants are planted or the seeds are sown, which cleans the field of weeds that are killed by the HPPD inhibitor, allowing for no-till practices, followed by planting or sowing of the soybeans in that same pre-treated field later on (burndown application using an HPPD inhibitor herbicide). The residual activity of IFT will also protect the emerging and growing soybean plants from competition by weeds in the early growth stages. Once the soybean plants have a certain size, and weeds tend to re-appear, glyphosate, or an HPPD inhibitor-glyphosate mixture, can be applied as post-emergent herbicide over the top of the plants.

In another embodiment of this invention, a field in which seeds containing the EE-GM3 event were sown, can be treated with an HPPD inhibitor herbicide, such as IFT, before the soybean plants emerge but after the seeds are sown (the field can be made weed-free before sowing using other means, typically conventional tillage practices such as ploughing, chissel ploughing, or seed bed preparation), where residual activity will keep the field free of weeds killed by the herbicide so that the emerging and growing soybean plants have no competition by weeds (pre-emergence application of an HPPD inhibitor herbicide). Once the soybean plants have a certain size, and weeds tend to re-appear, glyphosate—or an HPPD inhibitor-glyphosate mixture—can be applied as post-emergent herbicide over the top of the plants.

In another embodiment of this invention, plants containing the EE-GM3 event, can be treated with an HPPD inhibitor herbicide, such as IFT, over the top of the soybean plants (that have emerged from the seeds that were sown), which cleans the field of weeds killed by the HPPD inhibitor, which application can be together with (e.g., in a spray tank mix), followed by or preceded by a treatment with glyphosate as post-emergent herbicide over the top of the plants (post-emergence application of an HPPD inhibitor herbicide (with or without glyphosate)).

Also, in accordance with the current invention, soybean plants harboring EE-GM3 and EE-GM1 or EE-GM2 may be treated with the following insectides, herbicides or fungicides or soybean seeds harboring EE-GM3 and EE-GM1 or EE-GM2 may be coated with a seed coating comprising the following insectides, herbicides or fungicides:

Soybean Herbicides:

Alachlor, Bentazone, Trifluralin, Chlorimuron-Ethyl, Cloransulam-Methyl, Fenoxaprop, Fomesafen, Fluazifop, Glyphosate, Imazamox, Imazaquin, Imazethapyr, (S-)Metolachlor, Metribuzin, Pendimethalin, Tepraloxydim, Isoxaflutole, Glufosinate.

Soybean Insecticides:

Lambda-cyhalothrin, Methomyl, Parathion, Thiocarb, Imidacloprid, Clothianidin, Thiamethoxam, Thiacloprid, Acetamiprid, Dinetofuran, Flubendi amide, Rynaxypyr, Cyazypyr, Spinosad, Spinotoram, Emamectin-Benzoate, Fipronil, Ethiprole, Deltamethrin, β-Cyfluthrin, gamma and lambda Cyhalothrin, 4-[[(6-Chlorpyridin-3-yl)methyl](2,2-difluorethyl)amino]furan-2(5H)-on, Spirotetramat, Spinodiclofen, Triflumuron, Flonicamid, Thiodicarb, beta-Cyfluthrin.

Soybean Fungicides:

Azoxystrobin, Cyproconazole, Epoxiconazole, Flutriafol, Pyraclostrobin, Tebuconazole, Trifloxystrobin, Prothioconazole, Tetraconazole.

The following examples describe the identification of elite events EE-GM3, EE-GM1 and EE-GM2 and of plants containing a stack of event EE-GM3 with EE-GM1 or EE-GM3 with EE-GM2 and the development of tools for the specific identification of elite event EE-GM3, EE-GM1 or EE-GM2 and stacks thereof in biological samples.

Unless stated otherwise in the Examples, all recombinant techniques are carried out according to standard protocols as described in "Sambrook J and Russell D W (eds.) (2001) Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, New York" and in "Ausubel F A, Brent R, Kingston R E, Moore D D, Seidman J G, Smith J A and Struhl K (eds.) (2006) Current Protocols in Molecular Biology. John Wiley & Sons, New York".

Standard materials and references are described in "Croy R D D (ed.) (1993) Plant Molecular Biology LabFax, BIOS Scientific Publishers Ltd., Oxford and Blackwell Scientific Publications, Oxford" and in "Brown T A, (1998) Molecular Biology LabFax, 2nd Edition, Academic Press, San Diego". Standard materials and methods for polymerase chain reactions (PCR) can be found in "McPherson M J and WØller S G (2000) PCR (The Basics), BIOS Scientific Publishers Ltd., Oxford" and in "PCR Applications Manual, 3rd Edition (2006), Roche Diagnostics GmbH, Mannheim or www.roche-applied-science.com".

It should be understood that a number of parameters in any lab protocol such as the PCR protocols in the below Examples may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA or the selection of other primers in a PCR method may dictate other optimal conditions for the PCR protocol. These adjustments will however be apparent to a person skilled in the art, and are furthermore detailed in current PCR application manuals.

In the description and examples, reference is made to the following sequences:

SEQ ID No. 1: SalI fragment nucleotide sequence of vector pSF10.
SEQ ID No. 2: nucleotide sequence comprising the 5' region flanking the foreign DNA comprising the herbicide tolerance genes in EE-GM3.
SEQ ID No. 3: nucleotide sequence comprising the 3' region flanking the foreign DNA comprising the herbicide tolerance genes in EE-GM3.
SEQ ID No. 4: primer SOY028
SEQ ID No. 5: primer SOY029
SEQ ID No. 6: primer SMP187
SEQ ID No. 7: primer STV019
SEQ ID No. 8: nucleotide sequence of the amplicon
SEQ ID No. 9: primer 1 for amplification of control fragment (SOY01)
SEQ ID No. 10: primer 2 for amplification of control fragment (SOY02)
SEQ ID No. 11: nucleotide sequence of pB2/P35SAcK
SEQ ID No. 12: nucleotide sequence comprising the 5' region flanking the foreign DNA in EE-GM1
SEQ ID No. 13: nucleotide sequence comprising the 3' region flanking the foreign DNA in EE-GM1
SEQ ID No. 14: nucleotide sequence comprising the 5' region flanking the foreign DNA in EE-GM2
SEQ ID No. 15: nucleotide sequence comprising the 3' region flanking the foreign DNA in EE-GM2
SEQ ID No. 16: primer SOY06
SEQ ID No. 17: primer SOY07
SEQ ID No. 18: primer SOY09
SEQ ID No. 19: primer SOY010
SEQ ID No. 20: nucleotide sequence of a foreign DNA and plant flanking sequences in EE-GM3
SEQ ID No. 21: primer SHA130
SEQ ID No. 22: primer SHA178

EXAMPLES

1. Transformation of *Glycine max* with Herbicide Tolerance Genes

1.1. Description of the Foreign DNA Comprising the 2mEPSPS and HPPD-Pf-W336 Chimeric Genes

Plasmid pSF10 is a pUC19 derived cloning vector which contains a chimeric 2mepsps gene and a chimeric hppd-Pf-W336 gene located on a SalI fragment of about 7.3 kb. A full description of the DNA comprised between the two SalI restriction sites is given in Table 1 below. The nucleotide sequence is represented in SEQ ID No. 1.

TABLE 1

Nucleotide positions of the DNA comprised between the SalI restriction sites of pSF10 (SEQ ID No 1)

Nucleotide positions	Orientation	Description and references
188-479	complement	3'nos: sequence including the 3' untranslated region of the nopaline synthase gene from the T-DNA of pTiT37 of *Agrobacterium tumefaciens* (Depicker et al., 1982, Journal of Molecular and Applied Genetics, 1, 561-573)
480-1556	complement	hppdPf W336: the coding sequence of the 4-hydroxyphenylpyruvate dioxygenase of *Pseudomonas fluorescens* strain A32 modified by the replacement of the amino acid Glycine 336 with a Tryptophane, as described by Boudec et al. (2001) U.S. Pat. No. 6,245,968 B1

TABLE 1-continued

Nucleotide positions of the DNA comprised between the SalI restriction sites of pSF10 (SEQ ID No 1)

Nucleotide positions	Orientation	Description and references
1557-1928	complement	TPotp Y: coding sequence of an optimized transit peptide derivative (position 55 changed into Tyrosine), containing sequence of the RuBisCO small subunit genes of *Zea mays* (corn) and *Helianthus annuus* (sunflower), as described by Lebrun et al. (1996) U.S. Pat. No. 5,510,471
1929-2069	complement	5'tev: sequence including the leader sequence of the tobacco etch virus as described by Carrington and Freed (1990) Journal of Virology, 64, 1590-1597
2070-3359	complement	Ph4a748 ABBC: sequence including the promoter region of the histone H4 gene of *Arabidopsis thaliana*, containing an internal duplication (Chabouté et al., 1987) Plant Molecular Biology, 8, 179-191.
3360-4374		Ph4a748: sequence including the promoter region of the histone H4 gene of *Arabidopsis thaliana* (Chabouté et al., 1987) Plant Molecular Biology, 8, 179-191.
4375-4855		intron1 h3At: first intron of gene II of the histone H3.III variant of *Arabidopsis thaliana* (Chaubet et al., 1992) Journal of Molecular Biology, 225, 569-574.
4856-5227		TPotp C: coding sequence of the optimized transit peptide, containing sequence of the RuBisCO small subunit genes of *Zea mays* (corn) and *Helianthus annuus* (sunflower), as described by Lebrun et al. (1996) U.S. Pat. No. 5,510,471
5228-6565		2mepsps: the coding sequence of the double-mutant 5-enol-pyruvylshikimate-3-phosphate synthase gene of *Zea mays* (corn) (Lebrun et al., 1997) WO9704103-A 1
6566-7252		3'histonAt: sequence including the 3' untranslated region of the histone H4 gene of *Arabidopsis thaliana* (Chabouté et al., 1987) Plant Molecular Biology, 8, 179-191.

1.2. Event EE-GM3

The HPLC purified SalI-linearized pSF10 fragment of about 7.3 kb (containing the 2mEPSPS glyphosate-tolerance gene and the HPPD inhibitor tolerance gene HPPD-Pf-W336) was used to obtain transformed soybean plants by means of direct gene transfer into cells of soybean type Jack (Nickell, C. D., G. R. Noel, D. J. Thomas, and R. Waller. Registration of 'Jack' soybean. *Crop Sci* 1365. 30.1990), followed by regeneration of transformed plant cells into transgenic fertile soybean plants.

1.2.1 Identification of Elite Event EE-GM3

Elite event EE-GM3 was selected based on an extensive selection procedure based on good expression and stability of the herbicide tolerance genes, and its compatibility with optimal agronomic characteristics such as plant height, height to node, stand, vigor, seed yield, were evaluated. Soybean plants containing this event were selected from a wide range of different transformation events obtained using the same chimeric genes. Parameters used in the selection of this event were: a) acceptable tolerance to isoxaflutole herbicide application in field trials, b) acceptable tolerance to glyphosate herbicide application in field trials, c) acceptable tolerance to combined application of isoxaflutole and glyphosate herbicides in field trials, d) an insertion of the herbicide tolerance transgenes at a single locus in the soybean plant genome, with absence of vector backbone, e) overall agronomy similar to the parent plants used for

transformation (maturity, lodging, disease susceptibility, etc.), and f) no significant yield drag caused by the insertion of the transforming DNA (as compared to an isogenic line without the event, such as the plant line used for transformation, grown under the same conditions).

At the T3 generation, a homozygous line of the soybean transformation event EE-GM3 was selected for seed production. Multi-location replicated agronomic field studies were conducted in the regions of adaptation of the parent variety, Jack. Field evaluations included herbicide tolerance and agronomic performance. The agronomic performance of plants containing EE-GM3 was found comparable to Jack (when no herbicides were applied).

The field evaluations also showed that plants carrying the EE-GM3 event have:

- similar plant morphology and seed characteristics compared to Jack,
- no change in response to soybean diseases compared to Jack, and
- no changes in seed germination or dormancy compared to Jack.

Seed (T1 or S1 generation) harvested from the initial transformant (T0) plant (the plant transformed with the construct so as to produce event EE-GM3) in the greenhouse were planted in the field. Three blocks were planted and sprayed with 0, 2, or 4 kg/ha glyphosate. Seed was harvested from plants demonstrating the desired level of tolerance to the herbicide, glyphosate.

Seeds (T2 generation) harvested from self-pollinated T1 plants grown in the field were sown "plant to row". Chi square analysis of segregation data for rows (fully or partially tolerant) and of individual plants within rows (tolerant or sensitive) demonstrates the expected Mendelian inheritance of a single insertion for EE-GM3.

Selection and seed increase continued until a line was determined to be homozygous for transformation event EE-GM3 and selected for core seed production in the fourth generation. Then, T5 generation seed served as a candidate for the development of different varieties. Plants in the sixth generation (T6 generation) were crossed with conventional soybean breeding lines in an introgression program designed to move the event into a broader base of commercial soybean germplasm. F1 hybrid plants (EE-GM3 lines× conventional lines) were grown to maturity and the F2 seed was planted. Leaf samples of 901 F2 plants were analyzed by PCR primers designed to identify the zygosity of the EE-GM3 insert. The expected ratio of 1:2:1 for a single insertion segregation by the rules of Mendel was observed.

The selected event EE-GM3 was introduced in different commercial genetic backgrounds, and results of field trials on different locations were compared. Plants were challenged with glyphosate herbicide and/or isoxaflutole herbicide using different treatments. The plants exhibited good herbicide tolerance. Hundreds of different soybean cultivars containing event EE-GM3 were used in an inheritance study, and herbicides were applied. Selected lines from this trial were later increased in the field and also treated with herbicide. From that trial, 50 selected lines were increased, and these were also herbicide treated. The phytotoxicity scores for the latter lines sprayed with isoxaflutole and glyphosate showed some variability in response, but the range of responses among the lines reflected similar variability as was observed across 4 replications of the EE-GM3 event in the original Jack background, grown under the same treatment and environmental conditions. Hence, tolerance to the relevant herbicides across a broad range of germplasm was observed for plants comprising EE-GM3.

Furthermore, plants containing the event EE-GM3 had normal leaf, flower and pod morphology, excellent fertility, and showed no disease or abnormal insect susceptibility in multiple genetic backgrounds. During introgression into multiple genetic backgrounds no aberrant problems or abnormalities were observed.

In one season, a 10 location study was designed to compare the agronomic performance of double herbicide tolerant soybean comprising transformation event EE-GM3 to the transformation parent variety, Jack and some non-transgenic soybean varieties. Using a randomized complete block design, EE-GM3 plants were grown in replicated plots with either conventional weed control or with the intended herbicides, glyphosate and isoxaflutole. Plots with soybean plants containing transformation event EE-GM3 were sprayed with isoxaflutole herbicide at a target rate of 70 grams ai/Ha and with glyphosate herbicide at a target rate of 1060 grams ai/Ha. Herbicide application was made to these plants as a foliar spray at about the V4-V5 plant growth stage. Agronomic observations were made in the early, mid and late season. The plant density (parameter; stand count) was higher for the Jack and the non-transgenic variety plots than in the event EE-GM3 plots by one standard deviation. The early stand count difference may have been the result of seed lot quality, as the EE-GM3 planting seed was produced in counter season nursery, while the seed of the non-transgenic varieties was produced in the contiguous US, normal production season. However, the number of days to achieve 50% emergence and the plant vigor ratings were the same, indicating that the seed lots were comparable for these performance parameters. In the late season stand counts, Jack and the non-transgenic varieties remained different by one standard deviation from EE-GM3 plants. Plot yields of EE-GM3 event plants were also lower than those of Jack by one standard deviation, perhaps a result of the lower plant density of the EE-GM3 event plots. The yield of the non-transgenic varieties was more than Jack as could be expected because of the advancement in yield potential found in more recent varieties.

In one trial, plant health ratings were made at three growth stages: V4-5, R1 and full maturity. The first evaluation was shortly after the intended herbicide application. At the time of the final plant health evaluation, the EE-GM3-containing plants sprayed with both herbicides had the same score as the unsprayed Jack plants, or the unsprayed plants comprising EE-GM3. In ratings by the agronomic staff, the herbicide-sprayed plants received a health rating of 3-4 (moderate injury) at the V4-5 and R1 plant growth stages. The unsprayed plants (untransformed Jack or soybean plants containing EE-GM3) were rated as 4.6-4.8 (rating of 5 indicates no injury). At the final plant health rating, all the plots received the same rating of 5 (no injury).

One trial season was one of exceptional rainfall and crop injury in the EE-GM3 plants following the intended herbicide was more obvious than observed in other seasons. The field evaluations also included monitoring of the fitness characters (reproduction, disease resistance, fecundity, seed dispersal, dormancy, persistence). For the reproductive characteristics; days to emergence, days to 50% flowering and days to 90% pods maturing, the EE-GM3 and Jack plants were not different. No difference was noted in the reaction to natural infestations of plant diseases and insect pests. Although EE-GM3 produced less ultimate yield than Jack, no difference in fecundity (100 seed weight) was found. The assessment of seed dispersal parameters (pod shattering and plant lodging) found EE-GM3 and Jack to have the same pod shattering score, but found EE-GM3 plants to be less

prone to lodging. Evaluation of seed harvested from the 10 locations found no concerns raised by germination or dormancy testing.

In these trials during the season with exceptional rainfall, the final yield of EE-GM3 plants, regardless of the weed control treatment, was less than the yield of Jack by one standard deviation (perhaps a result of the lower plant density of the EE-GM3 event plots). In the exceptionally wet season, crop injury (bleaching in 10-30% of the crop area) was reported for EE-GM3 plots up to six weeks following foliar application of the glyphosate and isoxaflutole herbicides. However, by maturity, "no injury" plant health ratings were assigned to all the plots. Replicated multi-location field trials with EE-GM3 introgressed in elite soybean cultivar background, when compared to near-isogenic sister lines not containing the transgene, are expected to show no yield difference between plants containing event EE-GM3 and the near-isogenic lines (in the absence of herbicide treatment).

Further, in a replicated field trial significant crop tolerance (bleaching of less than 10%) was found in soybean plants comprising EE-GM3 when treated either pre- or post-emergence with IFT (70 gr ai/ha with 0.5% NIS, Agridex), but also significant crop tolerance (bleaching of less than 10%) was found in soybean plants comprising EE-GM3 when treated with a post-emergence application of pyrasulfotole (35 gr ai/ha with 0.5% NIS, Agridex), another HPPD inhibitor herbicide.

1.2.2 Identification of the Flanking Regions and Foreign DNA of Elite Event EE-GM3

The sequence of the regions flanking the foreign DNA comprising the herbicide tolerance genes in the EE-GM3 elite event was determined to be as follows:

1.2.2.1. Right (5') Flanking Region

The fragment identified as comprising the 5' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 2. The sequence between nucleotide 1 and 1451 corresponds to plant DNA, while the sequence between nucleotide 1452 and 1843 corresponds to foreign DNA.

1.2.2.2. Left (3') Flanking Region

The fragment identified as comprising the 3' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 3. The sequence between nucleotide 1 and 240 corresponds to foreign DNA, while the sequence between nucleotide 241 and 1408 corresponds to plant DNA.

1.2.2.3. Foreign DNA Comprising the Herbicide Tolerance Genes of EE-GM3

Using different molecular techniques, it has been determined that the foreign DNA of elite event EE-GM3 comprising the herbicide tolerance genes contains two partial 3' histonAt sequences in a head-to-head orientation, followed by 2 almost complete copies of the SalI fragment of pSF10 arranged in head-to-tail orientation (see FIG. **1**).

The foreign DNA comprising the herbicide tolerance genes of EE-GM3 thus contains in order the following sequences:

- from nucleotide 1 to nucleotide 199: the nucleotide sequence corresponding to complement of the nucleotide sequence of SEQ ID 1 from nt 6760 to nt 6958;
- from nucleotide 200 to nucleotide 624: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 1 from nt 6874 to nt 7298;
- from nucleotide 625 to nucleotide 7909: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 1 from nt 7 to nt 7291;
- from nucleotide 7910 to nucleotide 15163: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 1 from nt 12 to nt 7265; and
- from nucleotide 15164 to nucleotide 15187: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 3 from nt 217 to nt 240 (this sequence does not correspond to either pSF10 plasmid DNA or wt plant DNA and therefore is designated filler DNA).

This foreign DNA is preceded immediately upstream and contiguous with the foreign DNA by the 5' flanking sequence of SEQ ID No 2 from nucleotide 1 to 1451 and is followed immediately downstream and contiguous with the foreign DNA by the 3' flanking sequence of SEQ ID No 3 from nucleotide 241 to nucleotide 1408.

Confirmed full DNA sequencing of the foreign DNA and flanking DNA sequences in EE-GM3 resulted in the sequence reported in SEQ ID No. 20. In this sequence, the inserted DNA is from nucleotide position 1452 to nucleotide position 16638, and the 2 almost complete copies from pSF10 arranged in head-to-tail orientation are from nucleotide position 2257 to nucleotide position 16601. The 5' flanking DNA sequence in SEQ ID No. 20 is the sequence from nucleotide position 1 to nucleotide position 1451 in SEQ ID No. 20, and the 3' flanking DNA sequence in SEQ ID No. 20 is the sequence from nucleotide position 16639 to nucleotide position 17806 in SEQ ID No. 20.

1.3. Description of the Foreign DNA Comprising the Phosphinothricinacetyltransferase Chimeric Genes

Plasmid pB2/P35SAcK is a pUC19 derived cloning vector which contains a chimeric pat gene. A description of the vector is given in Table 2 below. The nucleotide sequence thereof is represented in SEQ ID No. 11.

TABLE 2

Nucleotide positions of constituents of pB2/P35SAcK (SEQ ID No 11)

Nucleotide positions	Description and references
461-1003	35S promotor from Cauliflower Mosaic Virus
1004-1011	Synthetic polylinker derived sequences
1012-1563	Synthetic pat gene (amino acid sequence from *Streptomyces viridochromogenes*)
1564-1581	Synthetic polylinker derived sequences
1582-1784	35S terminator from Cauliflower Mosaic Virus

1.4. Event EE-GM1

1.4.1 Identification of EE-GM1

Herbicide-resistant soybean was developed by transformation of soybean with vector pB2/P35SAcK using direct gene transfer.

Elite event EE-GM1 was selected based on an extensive selection procedure based on good expression and stability of the herbicide resistance gene and its compatibility with optimal agronomic characteristics.

1.4.2 Identification of the Flanking Regions and Foreign DNA of Elite Event EE-GM1

1.4.2.1. Right (5') Flanking Region

The fragment identified as comprising the 5' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 12. The sequence between nucleotide 1 and 209 corresponds to plant DNA, while the sequence between nucleotide 210 and 720 corresponds to foreign DNA.

1.4.2.2. Left (3') Flanking Region

The fragment identified as comprising the 3' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 13. The sequence between nucleotide

1 and 568 corresponds to foreign DNA, while the sequence between nucleotide 569 and 1000 corresponds to plant DNA.

1.4.2.3. Foreign DNA Comprising the Herbicide Tolerance Genes of EE-GM1

Using different molecular techniques, it has been determined that the foreign DNA of elite event EE-GM1 comprising the herbicide tolerance gene comprises two copies of the chimeric pat gene in a direct repeat structure (see FIG. **3**).

The foreign DNA comprising the herbicide tolerance genes of EE-GM1 thus contains in order the following sequences:

- from nucleotide 1 to nucleotide 3122: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 11 from nt 340 to nt 3461;
- from nucleotide 3123 to nucleotide 3458: the nucleotide sequence corresponding to the complement of the nucleotide sequence of SEQ ID 11 from nt 1 to nt 336;
- from nucleotide 3459 to nucleotide 4073: the nucleotide sequence corresponding to the complement of the nucleotide sequence of SEQ ID 11 from nt 3462 to nt 4076; and
- from nucleotide 4074 to nucleotide 6780: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 11 from nt 337 to nt 3043; and
- from nucleotide 6781 to nucleotide 6790: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 13 from nt 559 to nt 568 (this sequence does not correspond to either plasmid DNA or wt plant DNA and therefore is designated filler DNA).

This foreign DNA of EE-GM1 is preceded immediately upstream and contiguous with the foreign DNA at the 5' flanking sequence of SEQ ID No 12 from nucleotide 1 to 209 and is followed immediately downstream and contiguous with the foreign DNA at the 3' flanking sequence of SEQ ID No 13 from nucleotide 569 to nucleotide 1000.

1.5. Event EE-GM2

Herbicide-resistant soybean was developed by transformation of soybean with vector pB2/P35SAcK using direct gene transfer.

Elite event EE-GM2 was selected based on an extensive selection procedure based on good expression and stability of the herbicide resistance gene and its compatibility with optimal agronomic characteristics.

1.5.1. Identification of the Flanking Regions and Foreign DNA of Elite Event EE-GM2

1.5.1.1. Right (5') Flanking Region

The fragment identified as comprising the 5' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 14. The sequence between nucleotide 1 and 311 corresponds to plant DNA, while the sequence between nucleotide 312 and 810 corresponds to foreign DNA.

1.5.1.2. Left (3') Flanking Region

The fragment identified as comprising the 3' flanking region was sequenced and its nucleotide sequence is represented in SEQ ID No. 15. The sequence between nucleotide 1 and 507 corresponds to foreign DNA, while the sequence between nucleotide 569 and 1880 corresponds to plant DNA.

1.5.1.3. Foreign DNA Comprising the Herbicide Tolerance Genes of EE-GM2

Using different molecular techniques, it has been determined that the foreign DNA of elite event EE-GM2 comprising the herbicide tolerance gene comprises one copy of the chimeric pat gene (see FIG. **4**).

The foreign DNA comprising the herbicide tolerance genes of EE-GM2 thus contains in order the following sequences:

- from nucleotide 1 to nucleotide 391: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 11 from nt 3458 to nt 3848; and
- from nucleotide 392 to nucleotide 3436: the nucleotide sequence corresponding to the nucleotide sequence of SEQ ID 11 from nt 413 to nt 3457.

This foreign DNA of EE-GM2 is preceded immediately upstream and contiguous with the foreign DNA at the 5' flanking sequence of SEQ ID No 14 from nucleotide 1 to 311 and is followed immediately downstream and contiguous with the foreign DNA at the 3' flanking sequence of SEQ ID No 15 from nucleotide 508 to nucleotide 1880.

2. Development of Polymerase Chain Reaction Identification Protocols for EE-GM3

2.1. Primers

Specific primers were developed which recognize sequences within the elite event.

A primer was developed which recognizes a sequence within the 3' flanking region of EE-GM3. A second primer was then selected within the sequence of the foreign DNA so that the primers span a sequence of about 263 nucleotides. The following primers were found to give particularly clear and reproducible results in a PCR reaction on EE-GM3 DNA:

```
SOY028:
                                (SEQ ID No.: 4)
5'-ATC.gCT.TTA.ACg.TCC.CTC.Ag-3
(target: insert DNA)

SOY029:
                                (SEQ ID No.: 5)
5'-CAA.ggC.CTC.gAg.ATT.ATC-3'
(target: plant DNA)
```

Primers targeting an endogenous sequence are preferably included in the PCR cocktail. These primers serve as an internal control in unknown samples and in the DNA positive control. A positive result with the endogenous primer-pair (presence of an PCR amplified fragment of 413 bp) demonstrates that there is ample DNA of adequate quality in the genomic DNA preparation for a PCR product to be generated. The endogenous primers were selected to recognize the endogenous actin soybean gene:

```
SOY01
                                (SEQ ID No.: 9)
5'-gTC.AgC.CAC.ACA.gTg.CCT.AT-3'

SOY02
                                (SEQ ID No.: 10)
5'-gTT.ACC.gTA.CAg.gTC.TTT.CC-3'
```

2.2. Amplified Fragments

The expected amplified fragments in the PCR reaction are:

For primer pair SOY01-SOY02: 413 bp (endogenous control)

For primer pair SOY028-SOY029: 263 bp (EE-GM3 elite event)

2.3. Template DNA

Template DNA was prepared from a leaf punch according to Edwards et al. (Nucleic Acid Research, 19, p 1349, 1991). When using DNA prepared with other methods, a test run utilizing different amounts of template should be done. Usually 50 ng of genomic template DNA yields the best results.

2.4. Assigned Positive and Negative Controls

To avoid false positives or negatives, it was determined that the following positive and negative controls should be included in a PCR run:

Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result, no PCR products, is observed this indicates that the PCR cocktail was not contaminated with target DNA.

A DNA positive control (genomic DNA sample known to contain the transgenic sequences). Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.

A wild-type DNA control. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

2.5. PCR Conditions

Optimal results were obtained under the following conditions (In describing the various conditions for optimal results is meant to provide examples of such conditions. Clearly one skilled in the art could vary conditions, reagents and parameters such as using other Taq polymerases, and achieve desirable results):

the PCR mix for 25 μl reactions contains:

20 ng template DNA
2.5 μl 10× Amplification Buffer (supplied by the manufacturer with the Taq polymerase)
0.5 μl 10 mM dNTP's
0.4 μl SOY01 (10 pmoles/μl)
0.4 μl SOY02 (10 pmoles/μl)
0.7 μl SOY028 (10 pmoles/μl)
0.7 μl SOY029 (10 pmoles/μl)
0.1 μl Taq DNA polymerase (5 units/μl)
water up to 25 μl

the theimocycling profile to be followed for optimal results is the following:

4 min. at 95° C.
Followed by: 1 min. at 95° C.
1 min at 57° C.
2 min at 72° C.
For 5 cycles
Followed by: 30 sec. at 92° C.
30 sec. at 57° C.
1 min. at 72° C.
For 25 cycles
Followed by: 10 minutes at 72° C.

2.6. Agarose Gel Analysis

To optimally visualize the results of the PCR it was determined that between 10 and 20 μl of the PCR samples should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate molecular weight marker (e.g. 100 bp ladder Pharmacia).

2.7. Validation of the Results

It was determined that data from transgenic plant DNA samples within a single PCR run and a single PCR cocktail should not be acceptable unless 1) the DNA positive control shows the expected PCR products (transgenic and endogenous fragments), 2) the DNA negative control is negative for PCR amplification (no fragments) and 3) the wild-type DNA control shows the expected result (endogenous fragment amplification).

When following the PCR Identification Protocol for EE-GM3 as described above, lanes showing visible amounts of the transgenic and endogenous PCR products of the expected sizes, indicate that the corresponding plant from which the genomic template DNA was prepared, has inherited the EE-GM3 elite event. Lanes not showing visible amounts of either of the transgenic PCR products and showing visible amounts of the endogenous PCR product, indicate that the corresponding plant from which the genomic template DNA was prepared, does not comprise the elite event. Lanes not showing visible amounts of the endogenous and transgenic PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated. These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

2.8. Use of Discriminating PCR Protocol to Identify EE-GM3

Before attempting to screen unknowns, a test run, with all appropriate controls, is performed. The developed protocol might require optimization for components that may differ between labs (template DNA preparation, Taq DNA polymerase, quality of the primers, dNTP's, thermocyler, etc.).

Amplification of the endogenous sequence plays a key role in the protocol. One has to attain PCR and thermocycling conditions that amplify equimolar quantities of both the endogenous and the transgenic sequence in a known transgenic genomic DNA template. Whenever the targeted endogenous fragment is not amplified or whenever the targeted sequences are not amplified with the same ethidium bromide staining intensities, as judged by agarose gel electrophoresis, optimization of the PCR conditions may be required.

Leaf material from a number of soybean plants, some of which comprising EE-GM3 were tested according to the above-described protocol. Samples from elite event EE-GM3 and from soybean wild-type were taken as positive and negative controls, respectively.

FIG. **2** illustrates the result obtained with the elite event PCR Identification Protocol for EE-GM3 on a number of soybean plant samples. The samples in lanes 2 and 3 were found to contain elite event EE-GM3 as the 263 bp band is detected, while the samples in lanes 4 to 8 do not comprise EE-GM3. Lanes 6 and 7 comprise samples from other soybean transformation events obtained using the same herbicide tolerance chimeric genes; lane 8 contains DNA from wild type soybean plants and lane 9 represents the negative control (water) sample, lanes 1 and 10 represent the Molecular Weight Marker (100 bp ladder).

2.9. dPCR Assay for EE-GM3 Detection in Bulked Seed

A discriminating PCR (dPCR) assay is set up to detect low level presence of EE-GM3 in bulked seeds. A minimum level of 0.4% (w/w) of transgenic seeds in a bulk of non transgenic seeds was successfully detected under repeatable conditions. Therefore the Limit of Detection is determined to be 0.4% (w/w).

The following primers are applied in this target PCR reaction:

```
Forward primer targeted to the T-DNA sequence:
SHA130
                                    (SEQ ID No. 12)
5'-CTA.TAT.TCT.ggT.TCC.AAT.TTA.TC-3'

Reverse primer targeted to the 3' flanking
sequence:
SMP178
                                    (SEQ ID No. 13)
5'-TgA.ggC.ACg.TAT.TgA.TgA.CC-3'
```

The expected amplified fragment in the PCR reaction from these primers is 115 bp.

The target PCR reaction is performed on approximately 200 ng of template DNA prepared from ground bulked seed according to a modified Gentra Puregene DNA purification extraction kit (Qiagen). When using DNA prepared with other methods, a test run using samples with known relative levels of EE-GM3 should be performed.

A validated reference system PCR reaction, targeting an endogenous sequence, should ideally be performed in a separate PCR run to verify the suitability of the DNA sample for PCR analysis to avoid false negative results.

For unknown test samples the PCR experiment should ideally include the appropriate positive and negative control samples, i.e.:

Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result (no PCR product) is observed for both the target and the to reference system reaction this indicates that the PCR cocktail was not contaminated with target DNA.

A DNA positive control (genomic DNA sample known to contain the transgenic sequences). Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.

Also a wild-type DNA control can be added in this PCR. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

Optimal results are obtained under the following conditions:

the PCR mix for 25 μl reactions contains:

200 ng template DNA

5 μl 5× Reaction Buffer

0.25 μl 20 mM dNTP's

0.7 μl SHA130 (10 pmoles/l)

0.4 μl SMP178 (10 pmoles/l)

0.1 μl GO-Taq DNA polymerase (5 units/l)

Add water up to 25 ml

the thermocycling profile to be followed for optimal results is the following:

4 min. at 95° C.

Followed by: 1 min. at 95° C.

1 min. at 57° C.

2 min. at 72° C.

For 5 cycles

Followed by: 30 sec. at 92° C.

30 sec. at 57° C.

1 min. at 72° C.

For 30 cycles

Followed by: 10 minutes at 72° C.

To optimally visualize the results of the PCR it was determined that 25 μl of the PCR product should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate molecular weight marker (e.g. 50 bp ladder).

When following the PCR method as described above, lanes showing visible amounts of the target and reference system PCR products of the expected sizes, indicate that the test sample from which the genomic template DNA was prepared, contained levels of EE-GM3 elite event above the detection limit of the target reaction

Lanes not showing visible amounts of the target PCR products but showing visible amounts of the reference system PCR product, indicate that the test sample from which the genomic template DNA was prepared, contained levels of EE-GM3 elite event below the detection limit of the target reaction

Lanes not showing visible amounts of the endogenous and transgenic PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated.

These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

3. Use of a Specific Integration Fragment as a Probe for Detection of Material Comprising EE-GM3

A specific integration fragment of EE-GM3 is obtained by PCR amplification using specific primers SOY028 (SEQ ID No. 4) and SOY029 (SEQ ID No. 5) yielding an amplicon with the nucleotide sequence of SEQ ID No 8 or by chemical synthesis and is labeled. This integration fragment is used as a specific probe for the detection of EE-GM3 in biological samples. Nucleic acid is extracted from the samples according to standard procedures. This nucleic acid is then contacted with the specific probe under hybridization conditions which are optimized to allow formation of a hybrid. The formation of the hybrid is then detected to indicate the presence of EE-GM3 nucleic acid in the sample. Optionally, the nucleic acid in the samples is amplified using the specific primers prior to contact with the specific probe. Alternatively, the nucleic acid is labeled prior to contact with the specific probe instead of the integration fragment. Optionally, the specific probe is attached to a solid carrier (such as, but not limited to a filter, strip or beads), prior to contact with the samples.

4. Polymerase Chain Reaction Identification Protocol for EE-GM1

4.1. Primers

Specific primers were developed which recognize sequences within the elite event. More particularly, a primer was developed which recognizes a sequence within the 5'

flanking region of EE-GM1. A second primer was then selected within the sequence of the foreign DNA so that the primers span a sequence of about 183 nucleotides. The following primers were found to give particularly clear and reproducible results in a PCR reaction on EE-GM1 DNA:

```
SOY06:
                                (SEQ ID No.: 16)
5'-ggC.gTT.CgT.AgT.gAC.TgA.gg-3'
(target: plant DNA)

SOY07:
                                (SEQ ID No.: 17)
5'-gTT.TTA.CAA.CgT.CgT.gAC.Tgg-3'
(target: insert DNA)
```

Primers targeting an endogenous sequence are preferably included in the PCR cocktail. These primers serve as an internal control in unknown samples and in the DNA positive control. A positive result with the endogenous primer-pair demonstrates that there is ample DNA of adequate quality in the genomic DNA preparation for a PCR product to be generated. The endogenous primers were selected to recognize a housekeeping gene in *Glycine max*:

```
SOY01:
                                (SEQ ID No.: 9)
5'-gTC.AgC.CAC.ACA.gTg.CCT.AT-3'
(located in Glycine max actin 1 gene (Accession
J01298))

SOY02:
                                (SEQ ID No.: 10)
5'-gTT.ACC.gTA.CAg.gTC.TTT.CC-3'
(located in Glycine max actin 1 gene (Accession
J01298))
```

4.2. Amplified Fragments

The expected amplified fragments in the PCR reaction are:

For primer pair SOY01-SOY02: 413 bp (endogenous control)

For primer pair SOY06-SOY07: 183 bp (EE-GM1 elite Event)

4.3. Template DNA

Template DNA was prepared from a leaf punch according to Edwards et al. (Nucleic Acid Research, 19, p 1349, 1991). When using DNA prepared with other methods, a test run utilizing different amounts of template should be done. Usually 50 ng of genomic template DNA yields the best results.

4.4. Assigned Positive and Negative Controls

To avoid false positives or negatives, it was determined that the following positive and negative controls should be included in a PCR run:

- Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result, no PCR products, is observed this indicates that the PCR cocktail was not contaminated with target DNA.
- A DNA positive control (genomic DNA sample known to contain the transgenic sequences). Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.
- A wild-type DNA control. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

4.5. PCR Conditions

Optimal results were obtained under the following conditions:

the PCR mix for 25 μl reactions contains:

- 2.5 μl template DNA
- 2.5 μl 10× Amplification Buffer (supplied with Taq polymerase)
- 0.5 μl 10 mM dNTP's
- 0.5 μl SOY06 (10 pmoles/μl)
- 0.5 μl SOY07 (10 pmoles/μl)
- 0.25 μl SOY01 (10 pmoles/μl)
- 0.25 μl SOY02 (10 pmoles/μl)
- 0.1 μl Taq DNA polymerase (5 units/μl)
- water up to 25 μl

the thermocycling profile to be followed for optimal results is the following:

4 min. at 95° C.

Followed by: 1 min. at 95° C.

1 min. at 57° C.

2 min. at 72° C.

For 5 cycles

Followed by: 30 sec. at 92° C.

30 sec. at 57° C.

1 min. at 72° C.

For 25 cycles

Followed by: 5 minutes at 72° C.

4.6. Agarose Gel Analysis

To optimally visualize the results of the PCR it was determined that between 10 and 20 μl of the PCR samples should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate molecular weight marker (e.g. 100 bp ladder Pharmacia).

4.7. Validation of the Results

It was determined that data from transgenic plant DNA samples within a single PCR run and a single PCR cocktail should not be acceptable unless 1) the DNA positive control shows the expected PCR products (transgenic and endogenous fragments), 2) the DNA negative control is negative for PCR amplification (no fragments) and 3) the wild-type DNA control shows the expected result (endogenous fragment amplification).

When following the PCR Identification Protocol for EE-GM1 as described above, lanes showing visible amounts of the transgenic and endogenous PCR products of the expected sizes, indicate that the corresponding plant from which the genomic template DNA was prepared, has inherited the EE-GM1 elite event. Lanes not showing visible amounts of either of the transgenic PCR products and showing visible amounts of the endogenous PCR product, indicate that the corresponding plant from which the genomic template DNA was prepared, does not comprise the elite event. Lanes not showing visible amounts of the endogenous and transgenic PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated. These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

4.8. Use of Discriminating PCR Protocol to Identify EE-GM1

Before attempting to screen unknowns, a test run, with all appropriate controls, has to be performed. The developed protocol might require optimization for components that may differ between labs (template DNA preparation, Taq DNA polymerase, quality of the primers, dNTP's, thermocyler, etc.).

Amplification of the endogenous sequence plays a key role in the protocol. One has to attain PCR and thermocycling conditions that amplify equimolar quantities of both the endogenous and transgenic sequence in a known transgenic genomic DNA template. Whenever the targeted endogenous fragment is not amplified or whenever the targeted sequences are not amplified with the same ethidium bromide staining intensities, as judged by agarose gel electrophoresis, optimization of the PCR conditions may be required.

Glycine max leaf material from a number of plants, some of which comprising EE-GM1 were tested according to the above-described protocol. Samples from elite event EE-GM1 and from *Glycine max* wild-type were taken as positive and negative controls, respectively.

FIG. **5** illustrates the result obtained with the elite event PCR Identification Protocol for EE-GM1 on a number of soybean plant samples (lanes 1 to 14). The samples in lane 1 were found to contain the elite event as the 185 bp band is detected, while the samples in lanes 2, 3 and 4 do not comprise EE-GM1. Lane 2 comprises another soybean elite event, lane 3 represents a non-transgenic *Glycine max* control; lane 4 represents the negative control (water) sample, and lane 5 to represents the Molecular Weight Marker (100 bp).

5. Use of a Specific Integration Fragment as a Probe for Detection of Material Comprising EE-GM1

A specific integration fragment of EE-GM1 is obtained by PCR amplification using specific primers SOY06 (SEQ ID No. 16) and SOY07 (SEQ ID No. 17) or by chemical synthesis and is labeled. This integration fragment is used as a specific probe for the detection of EE-GM1 in biological samples. Nucleic acid is extracted from the samples according to standard procedures. This nucleic acid is then contacted with the specific probe under hybridization conditions which are optimized to allow formation of a hybrid. The formation of the hybrid is then detected to indicate the presence of EE-GM1 nucleic acid in the sample. Optionally, the nucleic acid in the samples is amplified using the specific primers prior to contact with the specific probe. Alternatively, the nucleic acid is labeled prior to contact with the specific probe instead of the integration fragment. Optionally, the specific probe is attached to a solid carrier (such as, but not limited to a filter, strip or beads), prior to contact with the samples.

6. Polymerase Chain Reaction Identification Protocol for EE-GM2

6.1. Primers

Specific primers were developed which recognize sequences within the elite event. More particularly, a primer was developed which recognizes a sequence within the 3' flanking region of EE-GM2. A second primer was then selected within the sequence of the foreign DNA so that the primers span a sequence of about 150 nucleotides. The following primers were found to give particularly clear and reproducible results in a PCR reaction on EE-GM2 DNA:

```
SOY9:
                                        (SEQ ID No.: 18)
5'-TgT.ggT.TAT.ggC.ggT.gCC.ATC-3'
(target: plant DNA)

SOY10:
                                        (SEQ ID No.: 19)
5'-TgC.TAC.Agg.CAT.CgT.ggT.gTC-3'
(target: insert DNA)
```

Primers targeting an endogenous sequence are preferably included in the PCR cocktail. These primers serve as an internal control in unknown samples and in the DNA positive control. A positive result with the endogenous primer-pair demonstrates that there is ample DNA of adequate quality in the genomic DNA preparation for a PCR product to be generated. The endogenous primers were selected to recognize a housekeeping gene in *Glycine max*:

```
SOY1:
                                        (SEQ ID No.: 9)
5'-gTC.AgC.CAC.ACA.gTg.CCT.AT-3'
(located in Glycine max actin 1 gene (Accession
J01298))

SOY2:
                                        (SEQ ID No.: 10)
5'-gTT.ACC.gTA.CAg.gTC.TTT.CC-3'
(located in Glycine max actin 1 gene (Accession
J01298))
```

6.2. Amplified Fragments

The expected amplified fragments in the PCR reaction are:

For primer pair SOY01-SOY02: 413 bp (endogenous control)

For primer pair SOY09-SOY010: 151 bp (EE-GM2 elite Event)

6.3. Template DNA

Template DNA was prepared from a leaf punch according to Edwards et al. (Nucleic Acid Research, 19, p 1349, 1991). When using DNA prepared with other methods, a test run utilizing different amounts of template should be done. Usually 50 ng of genomic template DNA yields the best results.

6.4. Assigned Positive and Negative Controls

To avoid false positives or negatives, it was determined that the following positive and negative controls should be included in a PCR run:

- Master Mix control (DNA negative control). This is a PCR in which no DNA is added to the reaction. When the expected result, no PCR products, is observed this indicates that the PCR cocktail was not contaminated with target DNA.
- A DNA positive control (genomic DNA sample known to contain the transgenic sequences). Successful amplification of this positive control demonstrates that the PCR was run under conditions which allow for the amplification of target sequences.
- A wild-type DNA control. This is a PCR in which the template DNA provided is genomic DNA prepared from a non-transgenic plant. When the expected result, no amplification of a transgene PCR product but amplification of the endogenous PCR product, is observed this indicates that there is no detectable transgene background amplification in a genomic DNA sample.

6.5. PCR Conditions

Optimal results were obtained under the following conditions:

the PCR mix for 25 μl reactions contains:

2.5 μl template DNA

2.5 μl 10× Amplification Buffer (supplied with Taq polymerase)

0.5 μl 10 mM dNTP's

0.5 μl SOY09 (10 pmoles/μl)

0.5 μl SOY010 (10 pmoles/μl)

0.25 μl SOY01 (10 pmoles/μl)

0.25 μl SOY02 (10 pmoles/μl)

0.1 μl Taq DNA polymerase (5 units/μl)

water up to 25 μl

the thermocycling profile to be followed for optimal results is the following:

4 min. at 95° C.

Followed by: 1 min. at 95° C.

1 min. at 57° C.

2 min. at 72° C.

For 5 cycles

Followed by: 30 sec. at 92° C.

30 sec. at 57° C.

1 min. at 72° C.

For 25 cycles

Followed by: 5 minutes at 72° C.

6.6. Agarose Gel Analysis

To optimally visualize the results of the PCR it was determined that between 10 and 20 μl of the PCR samples should be applied on a 1.5% agarose gel (Tris-borate buffer) with an appropriate molecular weight marker (e.g. 100 bp ladder Pharmacia).

6.7. Validation of the Results

It was determined that data from transgenic plant DNA samples within a single PCR run and a single PCR cocktail should not be acceptable unless 1) the DNA positive control shows the expected PCR products (transgenic and endogenous fragments), 2) the DNA negative control is negative for PCR amplification (no fragments) and 3) the wild-type DNA control shows the expected result (endogenous fragment amplification).

When following the PCR Identification Protocol for EE-GM2 as described above, lanes showing visible amounts of the transgenic and endogenous PCR products of the expected sizes, indicate that the corresponding plant from which the genomic template DNA was prepared, has inherited the EE-GM2 elite event. Lanes not showing visible amounts of either of the transgenic PCR products and showing visible amounts of the endogenous PCR product, indicate that the corresponding plant from which the genomic template DNA was prepared, does not comprise the elite event. Lanes not showing visible amounts of the endogenous and transgenic PCR products, indicate that the quality and/or quantity of the genomic DNA didn't allow for a PCR product to be generated. These plants cannot be scored. The genomic DNA preparation should be repeated and a new PCR run, with the appropriate controls, has to be performed.

6.8. Use of Discriminating PCR Protocol to Identify EE-GM2

Before attempting to screen unknowns, a test run, with all appropriate controls, has to be performed. The developed protocol might require optimization for components that may differ between labs (template DNA preparation, Taq DNA polymerase, quality of the primers, dNTP's, thermocyler, etc.).

Amplification of the endogenous sequence plays a key role in the protocol. One has to attain PCR and thermocycling conditions that amplify equimolar quantities of both the endogenous and transgenic sequence in a known transgenic genomic DNA template. Whenever the targeted endogenous fragment is not amplified or whenever the targeted sequences are not amplified with the same ethidium bromide staining intensities, as judged by agarose gel electrophoresis, optimization of the PCR conditions may be required.

Glycine max leaf material from a number of plants, some of which comprising EE-GM2 were tested according to the above-described protocol. Samples from elite event EE-GM2 and from *Glycine max* wild-type were taken as positive and negative controls, respectively.

FIG. **6** illustrates the result obtained with the elite event PCR Identification Protocol for EE-GM2 on a number of soybean plant samples (lanes 1 to 14). The samples in lane 1 were found to contain the elite event as the 185 hp band is detected, while the samples in lanes 2, 3 and 4 do not comprise EE-GM2. Lane 2 comprises another soybean elite event, lane 3 represents a non-transgenic *Glycine max* control; lane 4 represents the negative control (water) sample, and lane 5 represents the Molecular Weight Marker (100 bp).

7. Use of a Specific Integration Fragment as a Probe for Detection of Material Comprising EE-GM2

A specific integration fragment of EE-GM2 is obtained by PCR amplification using specific primers SOY09 (SEQ ID No. 18) and SOY010 (SEQ ID No. 19) or by chemical synthesis and is labeled. This integration fragment is used as a specific probe for the detection of EE-GM2 in biological samples. Nucleic acid is extracted from the samples according to standard procedures. This nucleic acid is then contacted with the specific probe under hybridization conditions which are optimized to allow formation of a hybrid. The formation of the hybrid is then detected to indicate the presence of EE-GM2 nucleic acid in the sample. Optionally, the nucleic acid in the samples is amplified using the specific primers prior to contact with the specific probe. Alternatively, the nucleic acid is labeled prior to contact with the specific probe instead of the integration fragment. Optionally, the specific probe is attached to a solid carrier (such as, but not limited to a filter, strip or heads), prior to contact with the samples.

8. Generation of Soybean Plants Comprising EE-GM3 and EE-GM1 or EE-GM3 and EE-GM2 and Assessment of Agronomic Performance of Such Plants

A soybean plant containing a combination of elite events EE-GM3 and EE-GM1 has been obtained by conventional crossing between a parent soybean plant comprising EE-GM3 and a parent soybean plant comprising EE-GM1. Progeny plants comprising both events are identified using PCR Identification Protocols for EE-GM1 and EE-GM3 as described herein. Specifically, crosses were made with plants containing EE-GM3 and several EE-GM1 lines. Resulting F1 plants were grown and sprayed with glyphosate and glufosinate. F2 seed was harvested from F1 plants and planted (F2 plants were not sprayed). F2 single plants were pulled. F2:F3 plant rows were grown and sprayed with glyphosate and glufosinate. Rows homozygous for both EE-GM3 and EE-GM1 were identified and later harvested.

Segregation data from this trial showed an expected Mendelian segregation of the events.

A soybean plant containing a combination of elite events EE-GM3 and EE-GM2 has been obtained by conventional crossing between a parent soybean plant comprising EE-GM3 and a parent soybean plant comprising EE-GM2. Progeny plants comprising both events are identified using PCR Identification Protocols for EE-GM2 and EE-GM3 as described herein. Specifically, crosses were made with plants containing EE-GM3 and plants containing EE-GM2. The resulting F1 plants were crossed to 4 conventional lines. The F1s from these crosses were grown in the field and sprayed with glyphosate and glufosinate. F2 seed harvested from F1 plants resistant to both herbicides was then planted. The F2 plants were sprayed with both glyphosate and glufosinate. Nine hundred plants tolerant to both herbicides were harvested and planted in a field trial. Expected Mendelian segregation data for the events was obtained in this trial. Approximately 40 lines homozygous for tolerance to both herbicides were selected for agronomic uniformity for further studies. These lines had good tolerance to both herbicides with good agronomic characteristics.

Field trials with such soybean plants comprising the combined events in different types of commercial germplasm are being conducted at multiple locations and various agronomic parameters of the plants are being evaluated, including plant height, height to node, stand, vigor, seed yield, and glyphosate, isoxaflutole and glufosinate tolerance levels.

9. Introgression of EE-GM3 and EE-GM1 or EE-GM2 into Preferred Cultivars

Elite event EE-GM3 and elite event EE-GM1 or EE-GM2 are introduced by repeated back-crossing into commercial soybean cultivars such as but not limited to Soybean Cultivar 7631014 (US2009252860); Soybean Cultivar 7431014 (US2009252859); Soybean Cultivar 7925084 (US2009252858); Soybean Cultivar 7311153 (US2009252857); Soybean Cultivar S070159 (US2009252856); Soybean Cultivar 7535357 (US2009246353); Soybean Cultivar S070160 (US2009246352); Soybean Cultivar 26074414 (US2009249508); Soybean Cultivar 7509171 (US2009249507); Soybean Cultivar S070158 (US2009246351); Soybean Cultivar 7511119 (US2009249506); Soybean Cultivar 7113111 (US2009238945); Soybean cultivar S06-02RM018047 (U.S. Pat. No. 7,592,518); Soybean Cultivar 7013345 (US2009232957); Soybean Cultivar 7041461 (US2009235376); Soybean Cultivar 7549450 (US2009232956); Soybean Cultivar 7317090 (US2009232955); Soybean Cultivar 2N2V58015 (US2009226597); Soybean Cultivar 7243182 (US2009226596); Soybean Cultivar 7143182 (US2009226595); Soybean Cultivar 7043182 (US2009220673); Soybean Cultivar S070157 (US2009222950); Soybean Cultivar 306924721 (US2009220672); Soybean Cultivar 7614385 (US2009220671); Soybean Cultivar 7925118 (US2009214750); Soybean Cultivar 7821295 (US2009214749); Soybean Cultivar 7811336 (US2009214748); Soybean Cultivar S070150 (US2009214747); Soybean Cultivar 6214260 (US2009214746); Soybean Cultivar S070152 (US2009214745); Soybean Cultivar 7429331 (US2009214751); Soybean Cultivar 26034631 (US2009208634); Soybean cultivar S07-03JR108674 (U.S. Pat. No. 7,560,621); Soybean cultivar S07-031KL016279 (U.S. Pat. No. 7,560,620); Soybean cultivar S06-CL959411 (U.S. Pat. No. 7,554,017); SOYBEAN CULTIVAR SG3870NRR (US2009158453); SOYBEAN CULTIVAR HFPR-G (CA2645702); Soybean cultivar S06-02JR423016 (U.S. Pat. No. 7,521,606); Soybean cultivar S06-01JR119814 (U.S. Pat. No. 7,518,039); Soybean cultivar S06-01JR119448 (U.S. Pat. No. 7,518,038); Soybean Cultivar 6540220 (US2009055960); Soybean Cultivar S060292 (US2009055959); Soybean Cultivar S050228 (US2009055958); Soybean cultivar S06-02JR423003 (U.S. Pat. No. 7,491,873); Soybean cultivar S06-02JR423005 (U.S. Pat. No. 7,491,872); Soybean cultivar S06-02JR409114 (U.S. Pat. No. 7,485,782); Soybean cultivar S06-SJ144056 (U.S. Pat. No. 7,473,823); Soybean cultivar (U.S. Pat. No. 7,470,835); Soybean cultivar 6910450 (US2008282369); SOYBEAN CULTIVAR 6223012 (U.S. Pat. No. 7,446,246); SOYBEAN CULTIVAR 6549250 (U.S. Pat. No. 7,446,245); Soybean Cultivar 17731225 (US2008271204); Soybean Cultivar 6928285 (US2008271203); Soybean Cultivar 6736054 (US2008271169); Soybean Cultivar S060299 (US2008271199); Soybean Cultivar S060294 (US2008271202); Soybean Cultivar 6943322 (US2008271201); Soybean cultivar 5343260 (US2008263719); Soybean cultivar 6439359 (US2008263704); Soybean cultivar 6238359 (US2008263703); Soybean cultivar 6547272 (US2008263702); Soybean cultivar 6929431 (US2008263701); Soybean cultivar 6703392 (US2008263700); Soybean cultivar 6044483 (US2008263699); Soybean cultivar S050224 (US2008263698); Soybean cultivar 6719022 (US2008263697); Soybean cultivar 5826056 (US2008263696); Soybean cultivar 6265047 (US2008263724); Soybean cultivar 6928331 (US2008263695); Soybean cultivar 6719331 (US2008263694); Soybean cultivar 6636454 (US2008263693); Soybean cultivar 6226454 (US2008263718); Soybean cultivar Q0073801 (US2008256657); SOYBEAN CULTIVAR 6326393 (US2008256652); SOYBEAN CULTIVAR 6408448 (US2008256651); Soybean cultivar 6449315 (US2008250524); Soybean cultivar S060296 (US2008250523); Soybean cultivar 6012078 (US2008250522); Soybean cultivar 6342078 (US2008250521); Soybean cultivar 6419156 (US2008250520); Soybean cultivar 5723264 (US2008250519); Soybean cultivar S050225 (US2008250518); Soybean cultivar S060298 (US2008244783); Soybean cultivar 6935331 (US2008244782); Soybean cultivar 6819456 (US2008244787); Soybean cultivar S060297 (US2008244781); Soybean cultivar 6135319 (US2008244786); Soybean cultivar 6819331 (US2008244780); Soybean cultivar 6137445 (US2008244779); Soybean cultivar 6917445 (US2008244778); Soybean cultivar 6111333 (US2008244777); Soybean Cultivar S050229 (US2008244776); Soybean Cultivar 6114011 (US2008244775); Soybean Cultivar 6900358 (US2008244784); Soybean Cultivar 6345184 (US2008244774); Soybean Cultivar 6836085 (US2008244773); Soybean Cultivar 6635047 (US2008244772); Soybean Cultivar 6139105 (US2008244771); SOYBEAN CULTIVAR 6434385

(US2008244770); SOYBEAN CULTIVAR 5060295 (US2008244769); Soybean Cultivar 6035184 (US2008244768); Soybean Cultivar S060293 (US2008209590); Soybean Cultivar 6733322 (US2008209594); SOYBEAN CULTIVAR 6421326 (US2008209593); Soybean Cultivar S060077 (US2008209589); SOYBEAN CULTIVAR 6600375 (US2008209592); Soybean cultivar S06-CL821457 (U.S. Pat. No. 7,420,104); Soybean cultivar S07-02KG294306 (U.S. Pat. No. 7,414,178); Soybean cultivar S06-BA046119 (U.S. Pat. No. 7,414,175); Soybean cultivar S07-02KG294307 (U.S. Pat. No. 7,411,114); Soybean Cultivar SG3865N (US2008189802); Soybean cultivar 6701475 (U.S. Pat. No. 7,408,097); Soybean Cultivar 1335025 (US2008178316); Soybean Cultivar 1686017 (US2008178315); Soybean Cultivar 2388028 (US2008178314); Soybean Cultivar 2387029 (US2008178313); Soybean cultivar S06-WW152330 (U.S. Pat. No. 7,388,129); Soybean cultivar 6424090 (U.S. Pat. No. 7,385,118); Soybean cultivar 6723322 (U.S. Pat. No. 7,385,115); Soybean cultivar SG4377NRR (U.S. Pat. No. 7,385,114); Soybean cultivar S06-02JR111334 (U.S. Pat. No. 7,381,864); Soybean cultivar 6141287 (U.S. Pat. No. 7,378,577); Soybean cultivar MT110501 (U.S. Pat. No. 7,378,576); Soybean cultivar (U.S. Pat. No. 7,378,575); Soybean cultivar S06-WW169267 (U.S. Pat. No. 7,375,261); Soybean cultivar 6223392 (U.S. Pat. No. 7,371,939); Soybean cultivar S06-CL968413 (U.S. Pat. No. 7,371,937); Soybean cultivar S06-CL951107 (U.S. Pat. No. 7,368,636); Soybean cultivar S06-MT9152077 (U.S. Pat. No. 7,361,810); Soybean Cultivar 4211676 (US2008092253); Soybean cultivar S06-M059029 (U.S. Pat. No. 7,355,101); Soybean Cultivar 6548193 (U.S. Pat. No. 7,345,228); Soybean cultivar 6440261 (U.S. Pat. No. 7,345,227); Soybean cultivar S060291 (U.S. Pat. No. 7,342,151); Soybean cultivar S06-MT9206166 (U.S. Pat. No. 7,339,094); Soybean cultivar S06-WW013107 (U.S. Pat. No. 7,339,093); Soybean cultivar S06-M03256 (U.S. Pat. No. 7,335,820); Soybean cultivar 6134466 (U.S. Pat. No. 7,332,656); Soybean cultivar S06-01JR123373 (U.S. Pat. No. 7,329,800); Soybean cultivar S06-MT9211059 (U.S. Pat. No. 7,326,831); Soybean cultivar 26170838 (US2008016590); Soybean cultivar 306612412 (US2008016588); Soybean cultivar 26660135 (US2008016587); Soybean cultivar 306734323 (US2008016586); Soybean cultivar S06-01JR122235 (U.S. Pat. No. 7,317,144); SOYBEAN CULTIVAR 5900450 (U.S. Pat. No. 7,314,986); Soybean cultivar S06-MT116260 (U.S. Pat. No. 7,314,984); Soybean cultivar S06-SJ143606 (U.S. Pat. No. 7,312,381); Soybean cultivar S06-98181-G01-35167 (U.S. Pat. No. 7,309,819); SOYBEAN CULTIVAR 26082635 (U.S. Pat. No. 7,304,219); Soybean cultivar BA922834 (U.S. Pat. No. 7,304,217); Soybean cultivar 01JR123480 (U.S. Pat. No. 7,304,216); Soybean cultivar M061422 (U.S. Pat. No. 7,304,215); Soybean cultivar 17082821 (US2007277262); Soybean cultivar 17621620 (US2007277261); Soybean cultivar 00977706 (US2007277260); Soybean cultivar S060182 (US2007277259); Soybean cultivar 26312034 (U.S. Pat. No. 7,301,078); Soybean cultivar 26143837 (U.S. Pat. No. 7,301,077); Soybean cultivar 435.TCS (US2007271626); Soybean cultivar 495.RC (US2007271625); Soybean cultivar 5306230 (U.S. Pat. No. 7,297,845); Soybean cultivar S06-WW167686 (U.S. Pat. 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It is observed that the introgression of the elite events into these cultivars does not significantly influence any of the desirable phenotypic or agronomic characteristics of these cultivars (no yield drag) while expression of the transgene, as determined by glyphosate and/or isoxaflutole or glufosinate tolerance, meets commercially acceptable levels.

The stacks may be advantageously further combined with one or more other soybean events available in the market, including but not limited to other herbicide tolerance events, such as events described in USDA-APHIS petitions: 09-349-01p, 09-201-01p, 09-183-01p, 09-082-01p, 09-015-01p, 06-354-01p, 06-271-01p, 06-178-01p, 98-238-01p, 98-014-01p, 97-008-01p, 96-068-01p, 95-335-01p, 93-258-01p, (see, e.g., www.aphis.usda.gov/brs/not_reg.html) or event MON89788 (Glyphosate tolerance) described in US 2006-282915, event DP-305423-1 (High oleic acid/ALS inhibitor tolerance) described in WO 2008/054747, MON87701 described in US2009130071, event 3560.4.3.5 described in US2009036308, or event DP-305423-1 described in US2008312082, or event BPS-CV127-9 (Event 127) of WO 2010/080829.

As used in the claims below, unless otherwise clearly indicated, the term "plant" is intended to encompass plant tissues, at any stage of maturity, as well as any cells, tissues, or organs taken from or derived from any such plant, including without limitation, any seeds, leaves, stems, flowers, roots, single cells, gametes, cell cultures, tissue cultures or protoplasts.

Reference seed comprising elite event EE-GM3 was deposited as 32-RRMM-0531 at the NCIMB (Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB9YA, Scotland) on Oct. 12, 2009, under NCIMB accession number NCIMB 41659, and the viability thereof was confirmed. An alternative name for EE-GM3 is event FG-072, or MST-FGØ72-3.

Reference seed comprising elite event EE-GM1 was deposited as 32RRMM0368 at the NCIMB (Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB9YA, Scotland) on Oct. 12, 2009, under NCIMB accession number NCIMB 41658. An alternative name for EE-GM1 is LL27, A2704-12, or ACS-GMØØ5-3.

Reference seed comprising elite event EE-GM2 was deposited as 32CON0688 at the NCIMB (Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB9YA, Scotland) on Oct. 12, 2009, under NCIMB accession number NCIMB 41660. An alternative name for EE-GM2 is LL55, A5547-127, or ACS-GMØØ6-4.

Reference seed comprising elite event EE-GM3 and EE-GM1 was deposited as 111606 soybean at the ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110-2209, USA) on Jun. 11, 2010, under ATCC accession number PTA-11041.

Reference seed comprising elite event EE-GM3 and EE-GM2 was deposited as 05SHX2XEB Soybean at the ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, Va. 20110-2209, USA) on Jun. 11, 2010, under ATCC accession number PTA-11042.

The above description of the invention is intended to be illustrative and not limiting.

Various changes or modifications in the embodiments described may occur to those skilled in the art. These can be made without departing from the spirit or scope of the invention.

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tttttatata tgttattagt taaaatttat taaaaatcat aaaattaagt aagtttcaca 180

taacatccaa tgattttctc gtaattttaa gactggacta aagaatatag tagtaacact 240

tctcttcaaa taatatactt tatttgcccg aggaatagca ttgccatatt gaactattag 300

gaaagctgaa catcaattgg tacacttgga tggttcccac ggtttattat tgtctacatc 360

tggtcatcca agcagaggtt atatcttcta taactcgaca aatcttcgtt gtgcctatat 420

agagttgctt gtacgactaa aacgcttata ataatcgtta tacaatctat gattcacagt 480

tatgatacgt gtatgcaata aatgaataga tagataaata tgatacaatt atacaattat 540

tctaaaatat atagaataca atatatgtat gtataaaaaa ttcataaaac accaataagc 600

atataattgc aattttgcaa aaccaaatta agaatataac tcaaatatta ctagaaacaa 660

aaaaaattat aaatcattgt cttcataaat taattctaag tatctacaaa tagaaataat 720

atgaatttta tataaaaaag taatataaat tttattcctt tcttaaattt atgaaaaata 780

atacttctat atttctatac atgtttctat acatgcgttt caatgtctga tagtgatagg 840

aaactctact gtattttcaa aagtttttt ttgtttaaat atattttttg tcatgtaatt 900

gtgtgtgttt tcatttacgt ccatgtaaaa agaaaatatt ttagttctat taaaatattt 960

tttttatttt ttatccttaa aatactttaa ataatatttt ttcctattta aagcattttt 1020

tataatttaa agcgctattt aaaacgtttt tagaataaaa acataaaaca aacacatttt 1080

aaaatgattg aaatgaaaaa taaaactaat gaaaacgaaa acaatactaa attacaggaa 1140

agaaaaatat attcaaactt ttatgtttaa aggtttttga atatttctct gattcgtttg 1200

aaatatgtga agaaaattaa aatatcaagt agtaggttac aacagttcgg gtgcaacagt 1260

gactatgaca gcaagataat agggccaata tatttggata cctctcttaa gacgtaaaca 1320

ttttgagcga gaaaataatg gaaaaaaaat aagtcattca aatgataata gatatataaa 1380

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attatattta tgtagctttg ctcattgtct tctacgggaa ctttcccgga cataggaacc 1500

gccctttcgt tatcctcatc catcgtgaaa tcaggaaata aatgttcgaa gatttgaggt 1560

caaaagtcga atttcatgtt gtctcttcta tttagataca aaattgaagc aattttcacc 1620

aatttaatgc caaaatttaa aacaacgctg tcctgatttc acgatggatg aggataacga 1680

aagggcggtt cctatgtccg ggaaagttcc cgtagaagac aatgagcaaa gctactgaaa 1740

cgcggacacg acgtcgcatt ggtacggata tgagttaaac cgactcaatt cctttattaa 1800

gacataaacc gattttggtt aaagtgtaac agtgagctga tat 1843

<210> SEQ ID NO 3
<211> LENGTH: 1408
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: 3' flanking sequence of foreign DNA comprising
herbicide tolerance genes in EE-GM3
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(240)
<223> OTHER INFORMATION: insert or foreign DNA
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (241)..(1408)
<223> OTHER INFORMATION: plant DNA

<400> SEQUENCE: 3

taacagtgag ctgatataaa accgaaacaa accggtacaa gtttgattga gcaacttgat 60

gacaaacttc agaattttgg ttattgaatg aaaatcatag tctaatcgta aaaaatgtac 120

agaagaaaag ctagagcaga acaaagattc tatattctgg ttccaattta tcatcgcttt 180

aacgtccctc agatttgatc gggctgcagg aattaatgtg gttcatccgt ctttttgtta 240

atgcggtcat caatacgtgc ctcaaagatt gccaaataga ttaatgtggt tcatctccct 300

atatgttttg cttgttggat tttgctatca catgtttatt gctccaaact aattataata 360

aaatgacttt caaatgattg gtgttgacat tcttttcaaa ttgttcgctg aagaaaagat 420

aatctcgagg ccttgatttg ttaatgcttt cattaataaa taaataaaat aactctttcc 480

aaatttcaat tcatgctttt atattgtgtg gttcatcctc atcttatgtc actattatca 540

tttcatgttt gagactttac ttggccatat ttgagaagac cttcttcatt ataggcaatt 600

ttatctccac aataatataa gagaatatct tgaattaata attattgagg atatattata 660

gggttctatg tggaactaaa gacatggtta ccccattaag agagagtata gaggaattac 720

ttttatttgc cacgaggcga cgcgacttgt atttattttg gaattgtact tttgcgtgag 780

cagtgtggct ctatgttggg gcctccactt gttggtgttt tatatatgtg aaaggaggat 840

gagggtgatg gttcatttct ttgcattatt tttgttattc gcgcgaatga tatatgccct 900

gtttttgaag attgataggg aagtccatat ataggaattg aagtgtcaaa agggtgtgag 960

tatgtgctat gataatcacc caattaatgt acatctggtg tggtgtttga atttgtaggt 1020

cattaattaa tattcctctt ggtgaagttt ggagttcttt tgcaattaca attctgtttt 1080

gtaagtgatt atgatggact tttagatgtt tctcaaacag taggtgtaaa gaaaaatggg 1140

ccctggtatg aaaatttgtt ttcactcttt ctcattcata tctttaaaaa aagaatgata 1200

attttgtaat aaaaataaaa aaatattaaa tattttctca aatcaaacaa cctttatttt 1260

ttatgccaac aataattttg ttaaagatgg agatttcaat tattatataa gagttcatta 1320

tagttgaaaa ttgaatgaat gtatatgttt acgtttttg tctcaagtga aactaagatc 1380

aaatattcat atctattgag ctggtctt 1408

<210> SEQ ID NO 4
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer SOY028

<400> SEQUENCE: 4

atcgctttaa cgtccctcag 20

<210> SEQ ID NO 5
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer SOY029

<400> SEQUENCE: 5

caaggcctcg agattatc 18

<210> SEQ ID NO 6
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer SMP187

<400> SEQUENCE: 6

atatcaaccc gtagctcgac 20

<210> SEQ ID NO 7
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer STV019

<400> SEQUENCE: 7

ggcattaaat tggtgaaaat tgc 23

<210> SEQ ID NO 8
<211> LENGTH: 263
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence of PCR amplicon using
primers SOY028 - SOY029

<400> SEQUENCE: 8

atcgctttaa cgtccctcag atttgatcgg gctgcaggaa ttaatgtggt tcatccgtct 60

ttttgttaat gcggtcatca atacgtgcct caaagattgc caaatagatt aatgtggttc 120

atctccctat atgttttgct tgttggattt tgctatcaca tgtttattgc tccaaactaa 180

ttataataaa atgactttca aatgattggt gttgacattc ttttcaaatt gttcgctgaa 240

gaaaagataa tctcgaggcc ttg 263

<210> SEQ ID NO 9
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer 1 for amplication of control fragment
(SOY01)

<400> SEQUENCE: 9

gtcagccaca cagtgcctat 20

<210> SEQ ID NO 10
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: primer 2 for amplication of control fragment
(SOY02)

<400> SEQUENCE: 10

gttaccgtac aggtctttcc 20

<210> SEQ ID NO 11
<211> LENGTH: 4076
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence of pB2/P35SAcK
<220> FEATURE:
<221> NAME/KEY: promoter
<222> LOCATION: (461)..(1003)
<223> OTHER INFORMATION: CamV35S promoter sequence
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1004)..(1011)
<223> OTHER INFORMATION: synthetic coding sequence for
phosphinoacetyltransferase
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1582)..(1784)
<223> OTHER INFORMATION: CaMV 35S terminator

<400> SEQUENCE: 11

tcgcgcgttt cggtgatgac ggtgaaaacc tctgacacat gcagctcccg gagacggtca 60

cagcttgtct gtaagcggat gccgggagca gacaagcccg tcagggcgcg tcagcgggtg 120

ttggcgggtg tcggggctgg cttaactatg cggcatcaga gcagattgta ctgagagtgc 180

accatatgca aacaaacata cacagcgact tatgctcaaa ttacaacggt atatatcctg 240

ccacatatgc ggtgtgaaat accgcacaga tgcgtaagga gaaaataccg catcaggcgc 300

cattcgccat tcaggctgcg caactgttgg gaagggcgat cggtgcgggc ctcttcgcta 360

ttacgccagc tggcgaaagg gggatgtgct gcaaggcgat taagttgggt aacgccaggg 420

ttttcccagt cacgacgttg taaaacgacg gccagtgaat tcccatggag tcaaagattc 480

aaatagagga cctaacagaa ctcgccgtaa agactggcga acagttcata cagagtctct 540

tacgactcaa tgacaagaag aaaatcttcg tcaacatggt ggagcacgac acgcttgtct 600

actccaaaaa tatcaaagat acagtctcag aagaccaaag ggcaattgag acttttcaac 660

aaagggtaat atccggaaac ctcctcggat tccattgccc agctatctgt cactttattg 720

tgaagatagt ggaaaaggaa ggtggctcct acaaatgcca tcattgcgat aaaggaaagg 780

ccatcgttga agatgcctct gccgacagtg gtcccaaaga tggacccccca cccacgagga 840

gcatcgtgga aaaagaagac gttccaacca cgtcttcaaa gcaagtggat tgatgtgata 900

tctccactga cgtaagggat gacgcacaat cccactatcc ttcgcaagac ccttcctcta 960

tataaggaag ttcatttcat ttggagagga cagggtaccc ggggatccac catgtctccg 1020

gagaggagac cagttgagat taggccagct acagcagctg atatggccgc ggtttgtgat 1080

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gattggacag ttgagagtac tgtttacgtg tcacataggc atcaaaggtt gggcctagga 1320

tccacattgt acacacattt gcttaagtct atggaggcgc aaggttttaa gtctgtggtt 1380

gctgttatag gccttccaaa cgatccatct gttaggttgc atgaggcttt gggatacaca 1440

gcccggggta cattgcgcgc agctggatac aagcatggtg gatggcatga tgttggtttt 1500

tggcaaaggg attttgagtt gccagctcct ccaaggccag ttaggccagt tacccagatc 1560

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acccggggat cctctagagt cgacctgcag gcatgcaagc ttggcgtaat catggtcata   1860
gctgtttcct gtgtgaaatt gttatccgct cacaattcca cacaacatac gagccggaag   1920
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ctcactgccc gctttccagt cgggaaacct gtcgtgccag ctgcattaat gaatcggcca   2040
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ggccaggaac cgtaaaaagg ccgcgttgct ggcgtttttc cataggctcc gcccccctga   2280
cgagcatcac aaaaatcgac gctcaagtca gaggtggcga aacccgacag gactataaag   2340
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aagacacgac ttatcgccac tggcagcagc cactggtaac aggattagca gagcgaggta   2640
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ttgatccggc aaacaaacca ccgctggtag cggtggtttt tttgtttgca agcagcagat   2820
tacgcgcaga aaaaaaggat ctcaagaaga tcctttgatc ttttctacgg ggtctgacgc   2880
tcagtggaac gaaaactcac gttaagggat tttggtcatg agattatcaa aaaggatctt   2940
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cttaccatct ggccccagtg ctgcaatgat accgcgagac ccacgctcac cggctccaga   3180
tttatcagca ataaaccagc cagccggaag ggccgagcgc agaagtggtc ctgcaacttt   3240
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tggtatggct tcattcagct ccggttccca acgatcaagg cgagttacat gatcccccat   3420
gttgtgcaaa aaagcggtta gctccttcgg tcctccgatc gttgtcagaa gtaagttggc   3480
cgcagtgtta tcactcatgg ttatggcagc actgcataat tctcttactg tcatgccatc   3540
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gcggcgaccg agttgctctt gcccggcgtc aatacgggat aataccgcgc cacatagcag   3660
aactttaaaa gtgctcatca ttggaaaacg ttcttcgggg cgaaaactct caaggatctt   3720
accgctgttg agatccagtt cgatgtaacc cactcgtgca cccaactgat cttcagcatc   3780
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gggaataagg gcgacacgga aatgttgaat actcatactc ttcctttttc aatattattg   3900
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aagcatttat cagggttatt gtctcatgag cggatacata tttgaatgta tttagaaaaa 3960

taaacaaata ggggttccgc gcacatttcc ccgaaaagtg ccacctgacg tctaagaaac 4020

cattattatc atgacattaa cctataaaaa taggcgtatc acgaggccct ttcgtc 4076

<210> SEQ ID NO 12
<211> LENGTH: 720
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence comprising the 5' region
flanking the foreign DNA in EE-GM1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(209)
<223> OTHER INFORMATION: plant DNA
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (210)..(720)
<223> OTHER INFORMATION: foreign DNA

<400> SEQUENCE: 12

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ctttttcaaa cctgctccca ccatttcgag tcaagagata gataaataga cacatctcat 120

tgcaccgatc gggggcgttc gtagtgactg agggggtcaa agaccaagaa gtgagttatt 180

tatcagccaa gcattctatt cttcttatgt cggtgcgggc ctcttcgcta ttacgccagc 240

tggcgaaagg gggatgtgct gcaaggcgat taagttgggt aacgccaggg ttttcccagt 300

cacgacgttg taaaacgacg gccagtgaat tcccatggag tcaaagattc aaatagagga 360

cctaacagaa ctcgccgtaa agactggcga acagttcata cagagtctct tacgactcaa 420

tgacaagaag aaaatcttcg tcaacatggt ggagcacgac acgcttgtct actccaaaaa 480

tatcaaagat acagtctcag aagaccaaag ggcaattgag acttttcaac aaagggtaat 540

atccggaaac ctcctcggat tccattgccc agctatctgt cactttattg tgaagatagt 600

ggaaaaggaa ggtggctcct acaaatgcca tcattgcgat aaaggaaagg ccatcgttga 660

agatgcctct gccgacagtg gtcccaaaga tggacccccca cccacgagga gcatcgtgga 720

<210> SEQ ID NO 13
<211> LENGTH: 1000
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence comprising the 3' region
flanking the foreign DNA in EE-GM1
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(568)
<223> OTHER INFORMATION: foreign DNA
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (569)..(1000)
<223> OTHER INFORMATION: plant DNA

<400> SEQUENCE: 13

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ccgctgcgcc ttatccggta actatcgtct tgagtccaac ccggtaagac acgacttatc 120

gccactggca gcagccactg gtaacaggat tagcagagcg aggtatgtag gcggtgctac 180

agagttcttg aagtggtggc ctaactacgg ctacactaga agaacagtat ttggtatctg 240

cgctctgctg aagccagtta ccttcggaaa aagagttggt agctcttgat ccggcaaaca 300

aaccaccgct ggtagcggtg gtttttttgt ttgcaagcag cagattacgc gcagaaaaaa 360

aggatctcaa gaagatcctt tgatcttttc tacggggtct gacgctcagt ggaacgaaaa 420

ctcacgttaa gggattttgg tcatgagatt atcaaaaagg atcttcacct agatcctttt 480

aaattaaaaa tgaagtttta aatcaatcta aagtatatat gagtaaactt ggtctgacag 540

ttaccaatgc ttaatcagtg aggcaccttt aatctagatg atctgtctca actttaccaa 600

aagttttgag cacatgtttg gattcacctt aaataatcta aaatcacagc ttgtttgatc 660

ccaaaggagt taattctaag taaaattgat tgagttaaaa caattgtgtt agatagagaa 720

attttctttg aataaaaaca tctagacaca aatcatttca cttcaaaata attttaaaca 780

aaataaattt tgcaattcat tcatccaaac aaacatttga attaactata atcaattcaa 840

attgttacag tgtgttgcat tcatatcatt cctaataagt ctacattaaa gattaagagt 900

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acagtggcaa cggatcatcc ttgctgcaaa acatagcttt 1000

<210> SEQ ID NO 14
<211> LENGTH: 810
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence comprising the 5' region
flanking the foreign DNA in EE-GM2
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(311)
<223> OTHER INFORMATION: plant DNA
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (312)..(810)
<223> OTHER INFORMATION: foreign DNA

<400> SEQUENCE: 14

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gttgttcatg cttgcgctgc taatcccctt ttgtatgcga aaatcgggtt tgggtcgggt 120

cgggtcagcc caacacgacc taatttgtgt tacgaaaatt tcaacaaaaa aaaaaagtta 180

tcttccgcca ttatcgccat tccgccacga tcattaaggc tatggcggcc gcaatggcgc 240

cgccatatga aacccgcaat gccatcgcta tttggtggca tttttccaaa aacccgcaat 300

gtcataccgt catcgttgtc agaagtaagt tggccgcagt gttatcactc atggttatgg 360

cagcactgca taattctctt actgtcatgc catccgtaag atgcttttct gtgactggtg 420

agtactcaac caagtcattc tgagaatagt gtatgcggcg accgagttgc tcttgcccgg 480

cgtcaatacg ggataatacc gcgccacata gcagaacttt aaaagtgctc atcattggaa 540

aacgttcttc ggggcgaaaa ctctcaagga tcttaccgct gttgagatcc agttcgatgt 600

aacccactcg tgcacccaac tgatcttcag catcttttac tttcaccagc gtttctgggt 660

gagcaaaaac aggaaggcaa aatgccgcaa aaaagggaat aacgccaggg ttttcccagt 720

cacgacgttg taaaacgacg gccagtgaat tcccatggag tcaaagattc aaatagagga 780

cctaacagaa ctcgccgtaa agactggcga 810

<210> SEQ ID NO 15
<211> LENGTH: 1880
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: nucleotide sequence comprising the 3' region
flanking the foreign DNA in EE-GM2
<220> FEATURE:

<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(507)
<223> OTHER INFORMATION: foreign DNA
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (508)..(1880)
<223> OTHER INFORMATION: plant DNA

<400> SEQUENCE: 15

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tccatagttg cctgactccc cgtcgtgtag ataactacga tacgggaggg cttaccatct    180

ggccccagtg ctgcaatgat accgcgagac ccacgctcac cggctccaga tttatcagca    240

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cgcaacgttg ttgccattgc tacaggcatc gtggtgtcac gctcgtcgtt tggtatggct    420

tcattcagct ccggttccca acgatcaagg cgagttacat gatcccccat gttgtgcaaa    480

aaagcggtta gctccttcgg tcctccgatg gcaccgccat aaccacaatt taacaacttt    540

ataaatgact tagtatatta gcaatttatc ttgtcacatg cacatatttt ataactataa    600

taggagtttg agtttaaatg atgtaatgaa ttttggattg catgttgttt tgtactatat    660

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The invention claimed is:

1. A method for identifying the simultaneous presence of elite events EE-GM3 and EE-GM2 in biological samples, confirming seed purity in seed samples, or screening seeds for the presence of elite events EE-GM3 and EE-GM2 in samples in seed lots, which method comprises detection of an EE-GM3 specific region with a specific primer pair or probe which specifically recognizes the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3 and the inserted foreign DNA contiguous therewith, and detecting an EE-GM2 specific region with a specific primer pair or probe which specifically recognizes the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM2 and the inserted foreign DNA contiguous therewith.

2. The method of claim **1**, said method comprising amplifying two DNA fragments of between 50 and 1000 bp from a nucleic acid present in said biological samples using a first polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3, said 5' flanking region comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or the 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, the other primer of said primers recognizing a sequence within the foreign DNA of EE-GM3 comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240 or the other primer of said primers recognizing a sequence within the foreign DNA of EE-GM3 comprising the nucleotide sequence of SEQ ID No. 1 or its complement, or comprising the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or its complement, and using a second polymerase chain reaction with at least two primers, one of said primers recognizing the 5' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM2, said 5' flanking region comprising the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or the 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM2, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, the other primer of said primers recognizing a sequence within the foreign DNA of EE-GM2 comprising the nucleotide sequence of the complement of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507 or the other primer of said primers recognizing a sequence within the foreign DNA of EE-GM2 comprising the nucleotide sequence of SEQ ID No. 11 or its complement, whereby said first and second polymerase reaction may be sequential or simultaneous.

3. The method of claim **2**,

(a) wherein said primer recognizing the 5' flanking region of EE-GM3 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or said primer recognizing the 3'

flanking region of EE-GM3 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and said primer recognizing a sequence within the foreign DNA of EE-GM3 comprises 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240 or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or its complement, and wherein said primer recognizing the 5' flanking region of EE-GM2 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or said primer recognizing the 3' flanking region of EE-GM3 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, and said primer recognizing a sequence within the foreign DNA of EE-GM2 comprises 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507 or the nucleotide sequence of SEQ ID No. 11 or its complement; or

(b) wherein said primer recognizing the 5' flanking region of EE-GM3 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or said primer recognizing the 3' flanking region of EE-GM3 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and said primer recognizing a sequence within the foreign DNA of EE-GM3 comprises at its 3' end at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240 or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or its complement, and wherein said primer recognizing the 5' flanking region of EE-GM2 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or said primer recognizing the 3' flanking region of EE-GM2 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, and said primer recognizing a sequence within the foreign DNA of EE-GM2 comprises at its 3' end at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507 or the nucleotide sequence of SEQ ID No. 11 or its complement.

4. The method of claim **3**, wherein said EE-GM3 specific primers comprise the sequence of SEQ ID No. 5 and SEQ ID No. 4, respectively, or the sequence of SEQ ID No. 7 and SEQ ID No. 5 respectively, and said EE-GM2 specific primers comprise the sequence of SEQ ID No. 18 and SEQ ID No. 19 respectively.

5. The method of claim **4**, which method comprises amplifying an EE-GM3 specific fragment of about 263 or 706 bp using the EE-GM3 PCR Identification Protocol and an EE-GM2 specific fragment of about 151 bp.

6. A kit for identifying the simultaneous presence of elite event EE-GM3 and elite event EE-GM2 in biological samples, said kit comprising one primer recognizing the 5' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3, said 5' flanking region comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or one primer recognizing the 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and one primer recognizing a sequence within the foreign DNA comprising herbicide tolerance genes in EE-GM3, said foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240 or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or its complement, and wherein said kit further comprises one primer recognizing the 5' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM2, said 5' flanking region comprising the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or one primer recognizing the 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM2, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, and one primer recognizing a sequence within the foreign DNA comprising herbicide tolerance genes in EE-GM2, said foreign DNA comprising the nucleotide sequence of the complement of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507 or the nucleotide sequence of SEQ ID No. 11 or its complement, wherein:

at least one of said primers is labeled;

the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence;

at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides 240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos. 2 or 3;

at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with

said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions; or

the nucleotide sequence of at least one of said primers comprises the nucleotide sequence of a nucleic acid fused to a nucleic acid from another origin, or its complement.

7. The kit of claim **6**,

(a) wherein said primer recognizing said 5' flanking region of EE-GM3 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or said primer recognizing the 3' flanking region of EE-GM3 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and said primer recognizing a sequence within the foreign DNA of EE-GM3 comprises 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240 or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or its complement, and wherein said primer recognizing said 5' flanking region of EE-GM2 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or said primer recognizing the 3' flanking region of EE-GM2 comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, and said primer recognizing a sequence within the foreign DNA of EE-GM2 comprises 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507 or the nucleotide sequence of SEQ ID No. 11 or its complement; or

(b) wherein said primer recognizing said 5' flanking region of EE-GM3 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or said primer recognizing said 3' flanking region of EE-GM3 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and said primer recognizing a sequence within said foreign DNA of EE-GM3 comprises at its 3' end at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 or the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240 or the nucleotide sequence of SEQ ID No. 1 or its complement, or the nucleotide sequence of SEQ ID No. 20 from nucleotide position 1452 to nucleotide position 16638 or its complement, and wherein said primer recognizing said 5' flanking region of EE-GM2 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or said primer recognizing said 3' flanking region of EE-GM2 comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, and said primer recognizing a sequence within said foreign DNA of EE-GM2 comprises at its 3' end at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 or the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507 or the nucleotide sequence of SEQ ID No. 11 or its complement; or

(c) comprising a primer comprising the sequence of SEQ ID No. 4 and a primer comprising the sequence of SEQ ID No. 5 or comprising a primer comprising the sequence of SEQ ID No. 5 and a primer comprising the sequence of SEQ ID No. 7 and further comprising a primer comprising the sequence of SEQ ID No. 18 and a primer comprising the sequence of SEQ ID No. 19.

8. A set of primer pairs suitable for use in an EE-GM3 and EE-GM2 specific detection, comprising a first primer pair comprising a first primer which-specifically recognizes a sequence within the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM3, and a second primer which specifically recognizes a sequence within the foreign DNA contiguous with said 5' or 3' flanking region in EE-GM3, said 5' flanking region comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408, and said foreign DNA contiguous with said 5' flanking region in EE-GM3 comprising the nucleotide sequence of the complement of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1843 and said foreign DNA contiguous with said 3' flanking region in EE-GM3 comprising the nucleotide sequence of SEQ ID No. 3 from nucleotide 1 to nucleotide 240; and a second primer pair comprising a third primer which specifically recognizes a sequence within the 5' or 3' flanking region of foreign DNA comprising herbicide tolerance genes in EE-GM2, and a fourth primer which specifically recognizes a sequence within the foreign DNA contiguous with said 5' or 3' flanking region in EE-GM2, said 5' flanking region comprising the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311, said 3' flanking region comprising the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880, and said foreign DNA contiguous with said 5' flanking region in EE-GM2 comprising the complement of the nucleotide sequence of SEQ ID No. 14 from nucleotide 312 to nucleotide 810 and said foreign DNA contiguous with said 3' flanking region in EE-GM2 comprising the nucleotide sequence of SEQ ID No. 15 from nucleotide 1 to nucleotide 507, wherein:

at least one of said primers is labeled;

the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence;

at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides

240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos. 2 or 3;

at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions; or

the nucleotide sequence of at least one of said primers comprises the nucleotide sequence of a nucleic acid fused to a nucleic acid from another origin, or its complement.

9. The set of primer pairs of claim **8**,

(a) wherein said first primer comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408 and wherein said third primer comprises a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or a nucleotide sequence of 17 to 200 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880; or

(b) wherein said first primer comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 2 from nucleotide 1 to nucleotide 1451 or a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 3 from nucleotide 241 to nucleotide 1408 and wherein said third primer comprises at its extreme 3' end a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of SEQ ID No. 14 from nucleotide 1 to nucleotide 311 or a nucleotide sequence of at least 17 consecutive nucleotides selected from the nucleotide sequence of the complement of SEQ ID No. 15 from nucleotide 508 to nucleotide 1880.

10. A set of primers, wherein

a first primer comprises at its extreme 3' end the sequence of SEQ ID No. 5;

a second primer comprises at its extreme 3' end the sequence of SEQ ID No. 4;

a third primer comprises at its extreme 3' end the sequence of SEQ ID No. 18; and

a fourth primer comprises at its extreme 3' end the sequence of SEQ ID No. 19, wherein:

at least one of said primers is labeled;

the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence; or

at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions.

11. The method of claim **1**, which method comprises hybridizing a nucleic acid of biological samples with a first specific probe for EE-GM3 and with a second specific probe for EE-GM2.

12. The method of claim **11**,

(a) wherein the sequence of said first specific probe has at least 80% sequence identity with a sequence comprising part of the 5' flanking sequence or the 3' flanking sequence of EE-GM3 and part of the sequence of the foreign DNA contiguous therewith and wherein the sequence of said second specific probe has at least 80% sequence identity with a sequence comprising part of the 5' flanking sequence or the 3' flanking sequence of EE-GM2 and part of the sequence of the foreign DNA contiguous therewith; or

(b) wherein the sequence of said first specific probe has at least 80% sequence identity with SEQ ID No. 2 from nucleotide 1431 to 1472 or SEQ ID No. 3 from nucleotide 220 to 261, or the complement of said sequences and wherein the sequence of said second specific probe has at least 80% sequence identity with SEQ ID No. 14 from nucleotide 301 to 322 or SEQ ID No. 15 from nucleotide 497 to 518, or the complement of said sequences.

13. A kit for identifying the simultaneous presence of elite event EE-GM3 and elite event EE-GM2 in biological samples, said kit comprising a pair of specific probes for the identification of the simultaneous presence of elite event EE-GM3 and elite event EE-GM2, wherein a first specific probe is capable of hybridizing specifically to a region comprising a part of the 5' or 3' flanking region of EE-GM3 and a part of the foreign DNA contiguous therewith, and a second specific probe is capable of hybridizing specifically to a region comprising a part of the 5' or 3' flanking region of EE-GM2 and a part of the foreign DNA contiguous therewith.

14. The kit of claim **13**,

(a) wherein the sequence of said first specific probe has at least 80% sequence identity with a sequence comprising part of the 5' flanking sequence or the 3' flanking sequence of foreign DNA comprising herbicide tolerance genes in EE-GM3 and part of the sequence of the foreign DNA contiguous therewith and wherein the sequence of said second specific probe has at least 80% sequence identity with a sequence comprising part of the 5' flanking sequence or the 3' flanking sequence of foreign DNA comprising herbicide tolerance genes in EE-GM2 and part of the sequence of the foreign DNA contiguous therewith; or

(b) wherein the sequence of said first specific probe comprises a nucleotide sequence having at least 80% sequence identity with SEQ ID No. 2 from nucleotide 1441 to 1462 or SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences and wherein the sequence of said second specific probe has at least 80% sequence identity with SEQ ID No. 14 from nucleotide 301 to 322 or SEQ ID No. 15 from nucleotide 497 to 518, or the complement of said sequences.

15. A pair of specific probes for the identification of the simultaneous presence of elite event EE-GM3 and elite event EE-GM2 in biological samples, wherein a first specific probe is capable of hybridizing specifically to a region comprising a part of the 5' or 3' flanking region of EE-GM3 and a part of the foreign DNA contiguous therewith, and a

second specific probe is capable of hybridizing specifically to a region comprising a part of the 5' or 3' flanking region of EE-GM2 and a part of the foreign DNA contiguous therewith.

16. The pair of probes of claim **15**,

a) wherein said first probe comprises a nucleotide sequence having at least 80% sequence identity with a sequence comprising part of the 5' flanking sequence or the 3' flanking sequence of foreign DNA comprising herbicide tolerance genes in EE-GM3 and part of the sequence of the foreign DNA contiguous therewith, or the complement thereof, and said second probe comprises a nucleotide sequence having at least 80% sequence identity with a sequence comprising part of the 5' flanking sequence or the 3' flanking sequence of foreign DNA comprising herbicide tolerance genes in EE-GM2 and part of the sequence of the foreign DNA contiguous therewith, or the complement thereof; or

b) wherein said first probe has at least 80% sequence identity with SEQ ID No. 2 from nucleotide 1441 to 1462 or SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences and wherein said second probe has at least 80% sequence identity with SEQ ID No. 14 from nucleotide 301 to 322 or SEQ ID No. 15 from nucleotide 497 to 518, or the complement of said sequences; or

c) wherein said first probe comprises a nucleotide sequence being essentially similar to SEQ ID No. 2 from nucleotide 1441 to 1462 or SEQ ID No. 3 from nucleotide 230 to 251, or the complement of said sequences, and said second probe comprises a nucleotide sequence being essentially similar to SEQ ID No. 14 from nucleotide 301 to 322 or SEQ ID No. 15 from nucleotide 497 to 518, or the complement of said sequences.

17. A method of detecting the presence of elite events EE-GM3 and EE-GM2 in biological samples through hybridization with a substantially complementary labeled nucleic acid probe in which the probe:target nucleic acid ratio is amplified through recycling of the target nucleic acid sequence, said method comprising:

a) hybridizing said target nucleic acid sequence to a first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1452 to nucleotide 1469 or its complement or said first nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No. 3 from nucleotide 223 to nucleotide 240 or its complement;

b) hybridizing said target nucleic acid sequence to a second nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No. 2 from nucleotide 1434 to nucleotide 1451 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No. 3 from nucleotide 241 to nucleotide 258 or its complement, wherein said first and second oligonucleotide overlap by at least one nucleotide and wherein either said first or said second oligonucleotide is labeled to be said labeled nucleic acid probe;

c) cleaving only the labeled probe within the probe:target nucleic acid sequence duplex with an enzyme which causes selective probe cleavage resulting in duplex disassociation, leaving the target sequence intact;

d) recycling of the target nucleic acid sequence by repeating steps (a) to (c); and

e) detecting cleaved labeled probe, thereby determining the presence of said target nucleic acid sequence, and

f) hybridizing said target nucleic acid sequence to a third nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No. 14 from nucleotide 312 to nucleotide 329 or its complement or said third nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No. 15 from nucleotide 490 to nucleotide 507 or its complement;

g) hybridizing said target nucleic acid sequence to a fourth nucleic acid oligonucleotide comprising the nucleotide sequence of SEQ ID No. 14 from nucleotide 294 to nucleotide 311 or its complement or said labeled nucleic acid probe comprising the nucleotide sequence of SEQ ID No. 15 from nucleotide 508 to nucleotide 525 or its complement, wherein said third and fourth oligonucleotide overlap by at least one nucleotide and wherein either said third or said fourth oligonucleotide is labeled to be said labeled nucleic acid probe;

h) cleaving only the labeled probe within the probe:target nucleic acid sequence duplex with an enzyme which causes selective probe cleavage resulting in duplex disassociation, leaving the target sequence intact;

i) recycling of the target nucleic acid sequence by repeating steps (f) to (h); and

j) detecting cleaved labeled probe, thereby determining the presence of said target nucleic acid sequence.

18. The kit of claim **6**, wherein at least one of said primers is labeled.

19. The kit of claim **7**, wherein at least one of said primers is labeled.

20. The set of primers of claim **8**, wherein at least one of said primers is labeled.

21. The set of primers of claim **9**, wherein at least one of said primers is labeled.

22. The set of primers of claim **10**, wherein at least one of said primers is labeled.

23. The kit of claim **7**, wherein the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.

24. The kit of claim **7**, wherein the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.

25. The set of primers of claim **8**, wherein the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.

26. The set of primers of claim **9**, wherein the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.

27. The set of primers of claim **10**, wherein the 5' end of at least one of said primers comprises one or more mismatches or a nucleotide sequence unrelated to the 5' or 3' flanking sequence of foreign DNA sequence.

28. The kit of claim **6**, wherein at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides 240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos. 2 or 3.

29. The kit of claim **7**, wherein at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences

and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides 240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos. 2 or 3.

30. The set of primers of claim **8**, wherein at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides 240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos. 2 or 3.

31. The set of primers of claim **9**, wherein at least one of said primers comprises a nucleotide sequence at their 3' end spanning the joining region between the plant DNA derived sequences and the foreign DNA sequences, said joining region being at nucleotides 1451-1452 in SEQ ID No 2 or nucleotides 240-241 in SEQ ID No 3, provided that the 17 consecutive nucleotides at the 3' end are not derived exclusively from either the foreign DNA or plant-derived sequences in SEQ ID Nos. 2 or 3.

32. The kit of claim **6**, wherein at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions.

33. The kit of claim **7**, wherein at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions.

34. The set of primers of claim **8**, wherein at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions.

35. The set of primers of claim **9**, wherein at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions.

36. The set of primers of claim **10**, wherein at least one of said primers comprises a sequence which is between 80 and 100% identical to a sequence within the 5' or 3' flanking region of the elite event or the foreign DNA of the elite event, respectively, and said primer sequence comprises at least one mismatch with said 5' or 3' flanking region or said foreign DNA, provided the at least one mismatch still allows specific identification of the elite event with these primers under optimized PCR conditions.

37. The kit of claim **6**, wherein the nucleotide sequence of at least one of said primers comprises the nucleotide sequence of a nucleic acid fused to a nucleic acid from another origin, or its complement.

38. The kit of claim **7**, wherein the nucleotide sequence of at least one of said primers comprises the nucleotide sequence of a nucleic acid fused to a nucleic acid from another origin, or its complement.

39. The set of primers of claim **8**, wherein the nucleotide sequence of at least one of said primers comprises the nucleotide sequence of a nucleic acid fused to a nucleic acid from another origin, or its complement.

40. The set of primers of claim **9**, wherein the nucleotide sequence of at least one of said primers comprises the nucleotide sequence of a nucleic acid fused to a nucleic acid from another origin, or its complement.

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