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(54) Titre : EVENEMENT DP-073496-4 DE BRASSICA GAT ET COMPOSITIONS ET PROCEDES POUR L'IDENTIFIER ET/OU LE DETECTER

(54) Title: BRASSICA GAT EVENT DP-073496-4 AND COMPOSITIONS AND METHODS FOR THE IDENTIFICATION AND/OR DETECTION THEREOF

(57) Abrégé/Abstract:
Compositions and methods related to transgenic glyphosate tolerant Brassica plants are provided. Specifically, the present invention provides Brassica plants having a DP-073496-4 event which imparts tolerance to glyphosate. The Brassica plant harboring the DP-073496-4 event at the recited chromosomal location comprises genomic/transgene junctions within SEQ ID NO: 2 or with genomic/transgene junctions as set forth in SEQ ID NO: 12 and/or 13. The characterization of the genomic insertion site of the event provides for an enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof. Various methods and compositions for the identification, detection, and use of the event are provided.

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(54) **Title**: BRASSICA GAT EVENT DP-073496-4 AND COMPOSITIONS AND METHODS FOR THE IDENTIFICATION AND/OR DETECTION THEREOF

(57) **Abstract**: Compositions and methods related to transgenic glyphosate tolerant *Brassica* plants are provided. Specifically, the present invention provides *Brassica* plants having a DP-073496-4 event which imparts tolerance to glyphosate. The *Brassica* plant harboring the DP-073496-4 event at the recited chromosomal location comprises genomic/transgene junctions within SEQ ID NO: 2 or with genomic/transgene junctions as set forth in SEQ ID NO: 12 and/or 13. The characterization of the genomic insertion site of the event provides for an enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof. Various methods and compositions for the identification, detection, and use of the event are provided.

BRIEF SUMMARY OF THE INVENTION

Compositions and methods related to transgenic glyphosate-tolerant *Brassica* plants are provided. Specifically, the present invention provides *Brassica* plants containing a transgene which imparts tolerance to glyphosate. The event may be, for example, DP-073496-4. The *Brassica* plant harboring the transgene at the recited chromosomal location comprises unique genomic/transgene junctions having at least the polynucleotide sequence of SEQ ID NO: 2 or at least the polynucleotide sequence of SEQ ID NO: 12 and/or 13. Further provided are the seeds deposited as Patent Deposit Number PTA-11504 and plants, plant cells, plant parts, seed and plant products derived therefrom. Characterization of the genomic insertion site of DP-073496-4 or any other event comprising integration of the glyphosate-tolerance transgene provides for an enhanced breeding efficiency and enables the use of molecular markers to track the transgene insert in the breeding populations and progeny thereof. Various methods and compositions for the identification, detection, and use of the glyphosate-N-acetyltransferase ("GAT" or "glyat") transformation event in *Brassica* are provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows synthesis of plasmid PHP28181. Plasmid PHP28181 was used to produce the GAT *Brassica* lines.

Figure 2 provides a schematic map of plasmid PHP28181.

Figure 3 provides a schematic map of insertion DNA, fragment PHP28181A.

Figure 4 provides a schematic representation of fragment A from PHP28181 (PHP28181A), specifically a schematic map of *Hind* III/*Not* I fragment (PHP28181A) containing the *gat4621* gene cassette that was used for plant transformation to generate DP-073496-4 Brassica. The fragment size is 2112 bp. The construct-specific primer locations of 09-0-3290/09-0-3288 are indicated on the map.

Figure 5 Southern analysis of Construct Specific PCR of Leaf DNA From DP-073496-4 Brassica and Non-Genetically Modified Control Brassica. PCR amplification with primer set 09-0-3290/09-0-3288 targeting the unique ubiquitin promoter and *gat*4621 junction present in DP-073496-4canola. Expected PCR amplicon size is 675 bp.

Figure 6 Southern analysis of Brassica FatA gene PCR of leaf DNA from DP-073496-4 Brassica and Non-Genetically Modified Control Brassica. PCR amplification of endogenous brassica FatA gene with primer set 09-0-2812/09-02813 as positive control for PCR amplification. Expected PCR amplicon size is 506 bp.

in its genome in the following order: a polynucleotide comprising SEQ ID NO: 12, a polynucleotide encoding a glyphosate-N-acetyltransferase and a polynucleotide comprising SEQ ID NO: 13 is provided. The term "event DP-073496-4 specific" refers to a polynucleotide sequence which is suitable for discriminatively identifying event DP-073496-4 in plants, plant material, or in products such as, but not limited to, oil produced from the seeds, or food or feed products (fresh or processed) comprising, or derived from, plant material.

Compositions further include seed deposited as Patent Deposit Numbers PTA-11504 and plants, plant cells, and seed derived therefrom. Applicant(s) have made a deposit of at least 2500 seeds of *Brassica* event DP-073496-4 with the American Type Culture Collection (ATCC), Manassas, VA 20110-2209 USA on November 24, 2010 and the deposit will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. Deposits are made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The seeds deposited with the ATCC are taken from the deposit maintained by Pioneer Hi-Bred International, Inc., 7250 NW 62nd Avenue, Johnston, Iowa 50131-1000. Access to this deposit will be available during the pendency of the application to the Commissioner of Patents and Trademarks and persons determined by the Commissioner to be entitled thereto upon request. Upon allowance of any claims in the application, the Applicant(s) will make available to the public, pursuant to 37 C.F.R. §1.808, sample(s) of the deposit. The deposit of seed comprising *Brassica* event DP-073496-4 will be maintained in the ATCC depository, which is a public depository, for a period of 30 years or 5 years after the most recent request or for the enforceable life of the patent, whichever is longer and will be replaced if it becomes nonviable during that period. Additionally, Applicant(s) will have satisfied all the requirements of 37 C.F.R. §§1.801 - 1.809, including providing an indication of the viability of the sample upon deposit. Applicant(s) have no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicant(s) do not waive any infringement of their rights granted under this patent or rights applicable to event DP-073496-4 under the Plant Variety Protection Act (7 USC §2321, et seq.). Unauthorized seed multiplication prohibited. The seed may be regulated.

As used herein, the term "*Brassica*" means any *Brassica* plant and includes all plant varieties that can be bred with *Brassica*. As used herein, the term plant includes plant cells, plant organs, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps and plant cells that are

intact in plants or parts of plants such as embryos, pollen, ovules, seeds, leaves, flowers, branches, fruit,

Applicant's or agent's file reference	35718/39908	International application No.	PCT/US2010/

INDICATIONS RELATING TO DEPOSITED MICROORGANISM OR OTHER BIOLOGICAL MATERIAL

(PCT Rule 13*bis*)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 40, line 5

B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet ☐

Name of depository institution

American Type Culture Collection

Address of depositary institution *(including postal code and country)*

10801 University Blvd.
Manassas, Virginia 20110-2209 USA

Date of deposit	Accession Number
24 November 2010	PTA-11504

C. ADDITIONAL INDICATIONS *(leave blank if not applicable)* This information is continued on an additional sheet ☐

page 3, line 9; page 5, line 8; page 82, line 8; page 82, line 19; page 84, line 33; page 85, line 15 and page 85, line 21

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE *(if the indicators are not for all designated States)*

E. SEPARATE FURNISHING OF INDICATIONS *(leave blank if not applicable)*

The indications listed below will be submitted to the International Bureau later *(specify the general nature of the indications e.g., "Accession Number of Deposit")*

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Authorized officer	Authorized officer

Form PCT/RO/134 (July 1998)

THAT WHICH IS CLAIMED:

1. A brassica plant having in its genome in the following order: a polynucleotide comprising SEQ ID NO: 12, a polynucleotide encoding a glyphosate-N-acetyltransferase and a polynucleotide comprising SEQ ID NO: 13.

2. The brassica plant of claim 1, wherein said plant is a progeny of a plant grown from seeds of ATCC Seed Deposit PTA-11504.

3. A plant material derived from said brassica plant of claim 2.

4. The plant material of claim 3, wherein said plant material comprises a food product.

5 A transgenic seed having in its genome in the following order: a polynucleotide comprising SEQ ID NO: 12, a polynucleotide encoding a glyphosate-N-acetyltransferase and a polynucleotide comprising SEQ ID NO: 13.

6. A transgenic seed of ATCC Seed Deposit PTA-11504.

7. A plant material derived from the transgenic seed of claim 6.

8. The plant material of claim 7, wherein said plant material comprises a food product or an oil.

9. An isolated polynucleotide comprising SEQ ID NO: 12 and 13.

10. The isolated polynucleotide of claim 9, wherein said polynucleotide comprises a nucleotide sequence set forth in SEQ ID NO:2, 11, 14, 15, 16 17, 18 or 19.

11. A DNA detection kit comprising at least one polynucleotide that can specifically detect a DP-073496-4 specific region, wherein said polynucleotide comprises at least one DNA molecule of a sufficient length of contiguous nucleotides identical or complementary to SEQ ID NO:11.

12. The DNA detection kit of claim 11, wherein said polynucleotide that can specifically detect a DP-073496-4 specific region comprises a polynucleotide having SEQ ID NO:12 or 13.

13. The DNA detection kit of claim 11, wherein said polynucleotide comprises a sequence which hybridizes under stringent conditions with sequences comprising:

(a) the sequences of SEQ ID NO:8 and SEQ ID NO:10; and,

(b) the sequences of SEQ ID NO:9 and SEQ ID NO: 10.

14. A method for identifying event DP-073496-4 in a biological sample, comprising

(a) contacting said sample with a first and a second primer; and,

(b) amplifying a polynucleotide comprising a DP-073496-4 specific region.

15. The method of claim 14, wherein the polynucleotide comprising the DP-073496-4 specific region comprises SEQ ID NO:12, 14, or 16.

16. The method of claim 14, wherein the polynucleotide comprising the DP-073496-4 specific region comprises SEQ ID NO:13, 15, or 17.

17. The method of claim 14, 15 or 16, further comprising detecting the DP-073496-4 specific region.

18. The method of claim 14, 15, 16, or 17, said first primer comprises a first fragment of SEQ ID NO: 11 and the second primer comprises a second fragment of SEQ ID NO:11, wherein said first and said second primer flank said DP-073496-4 specific region and share sufficient sequence homology or complementarity to said polynucleotide to amplify said DP-073496-4 specific region.

19. The method of claim 18, wherein

a) said first primer comprises a fragment of SEQ ID NO:10 and said second primer comprises a fragment of SEQ ID NO:8;

b) said first primer comprises a fragment of SEQ ID NO:10 and said second primer comprises a fragment of SEQ ID NO:9; or,

c) said first primer comprises a fragment of SEQ ID NO:8 and said second primer comprises a fragment of SEQ ID NO:9.

20. The method of claim 18, wherein said first and or said second primer comprises at least 8 consecutive polynucleotides of SEQ ID NO: 11.

21. The method of claim 19, wherein said first and said second primer comprises at least 8 consecutive polynucleotides of SEQ ID NO:8, 10, or 9.

22. The method of claim 14, wherein the amplification of a polynucleotide comprising a DP-073496-4 specific region indicates seed purity or seed lot purity.

23. The method of claim 17, wherein the detection of said DNA amplicon molecule in said DNA amplification reaction indicates seed purity or seed lot purity.

24. A method of detecting the presence of DNA corresponding to a DP-073496-4 event in a sample, the method comprising:

(a) contacting the sample with a polynucleotide probe that hybridizes under stringent hybridization conditions with DNA from brassica event DP-073496-4 and specifically detects the DP-073496-4 event;

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

(c) detecting hybridization of the probe to the DNA, wherein detection of hybridization indicates the presence of the DP-073496-4 event.

25. The method of claim 24, wherein said sample comprises brassica tissue.

26. The method of claim 24 or 25, wherein the detection of hybridization indicates seed purity or seed lot purity.

27. A method of producing a glyphosate tolerant plant comprising breeding a brassica plant having in its genome in the following order: a polynucleotide comprising SEQ ID NO: 12, a polynucleotide encoding a glyphosate-N-acetyltransferase and a polynucleotide comprising SEQ ID NO: 13 or a plant that is a progeny of a plant grown from seeds of ATCC Seed Deposit PTA-11504,

and selecting progeny by analyzing for at least one polynucleotide comprising a DP-073496-4 specific region.

28. The method of claim 27, wherein said polynucleotide comprising the DP-073496-4 specific region comprises SEQ ID NO:12 or 13.

29. The method of claim 28, wherein said polynucleotide is selected from the group consisting of:

(a) a nucleotide sequence set forth in SEQ ID NO:11, 14, 15, 16, or 17;

(b) a nucleotide sequence comprising a fragment of SEQ ID NO: 11, 14, 15, 16, or 17.

30. A method for controlling weeds in an area of cultivation comprising applying an effective amount of glyphosate to the area of cultivation having a plant having in its genome in the following order: a polynucleotide comprising SEQ ID NO: 12, a polynucleotide encoding a glyphosate-N-acetyltransferase and a polynucleotide comprising SEQ ID NO: 13; or, a plant that is a progeny of a plant grown from seeds of ATCC Seed Deposit PTA-11504.

31 A method of growing a glyphosate tolerant brassica plant comprising

(a) planting in an area of cultivation a brassica plant or seed having in its genome a polynucleotide comprising SEQ ID NO: 12, a polynucleotide encoding a glyphosate-N-acetyltransferase and a polynucleotide comprising SEQ ID NO: 13, or a plant that is a progeny of a plant grown from seeds of ATCC Seed Deposit PTA-1504, and,

(b) applying glyphosate to the area of cultivation.

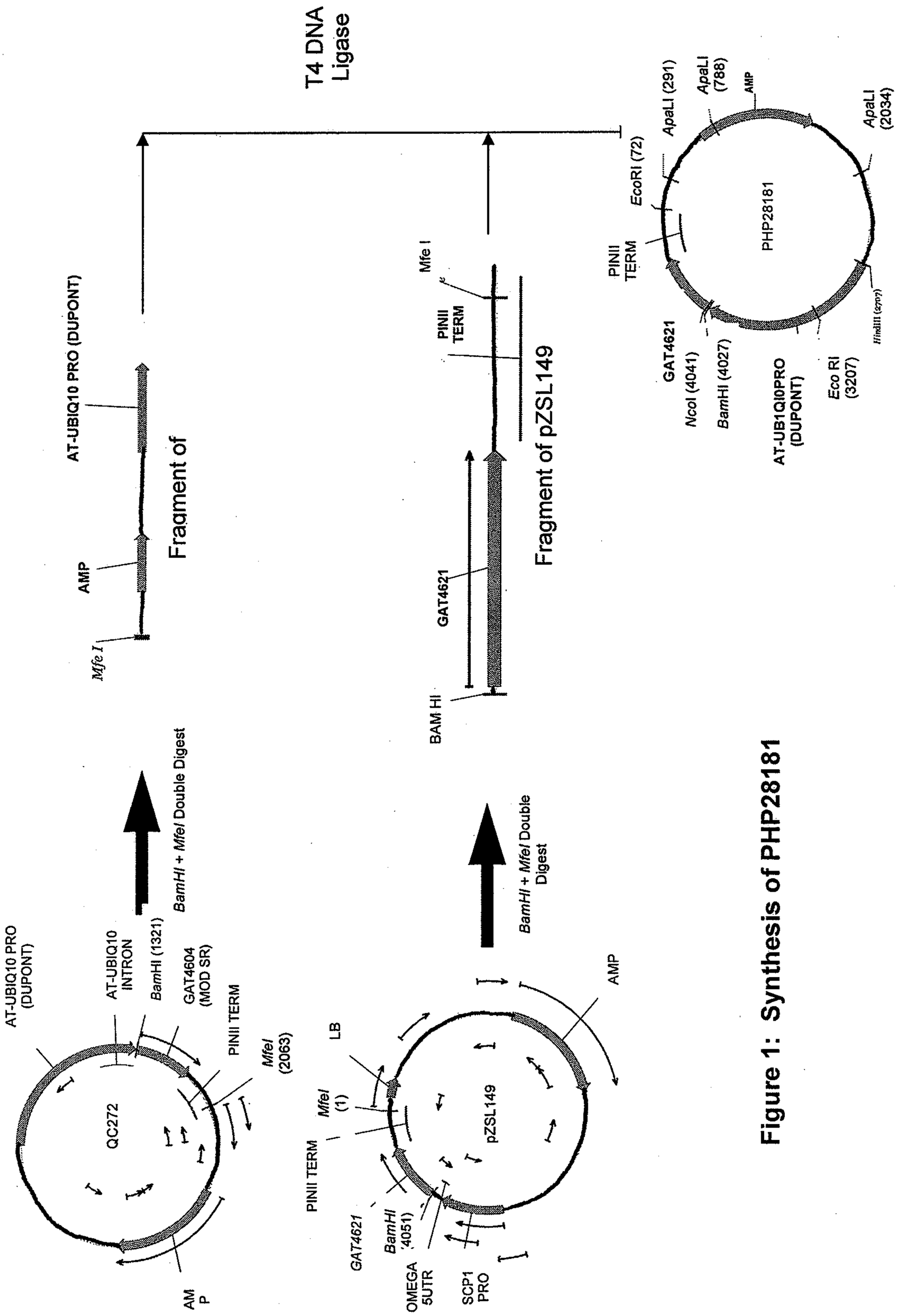


Figure 1: Synthesis of PHP28181

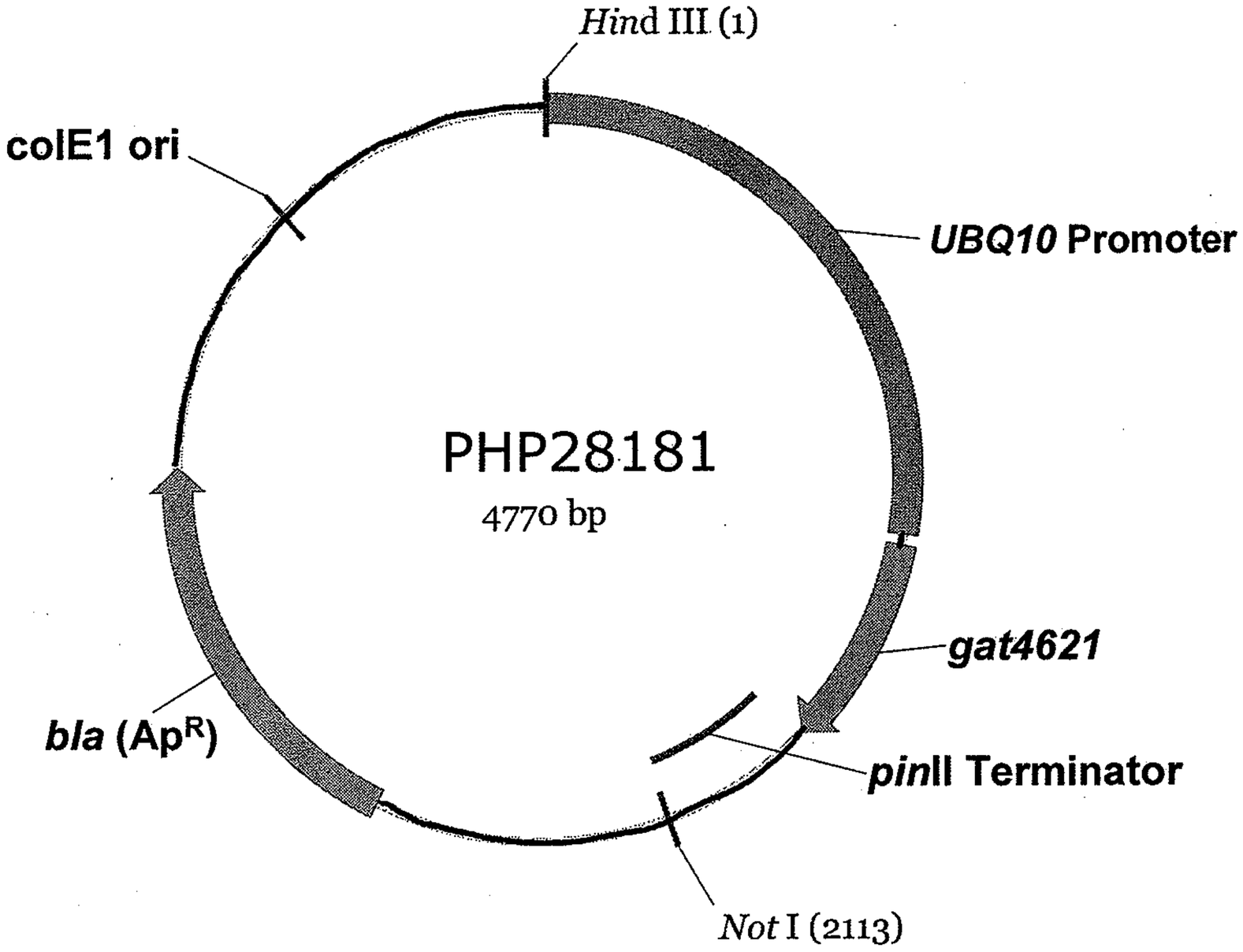
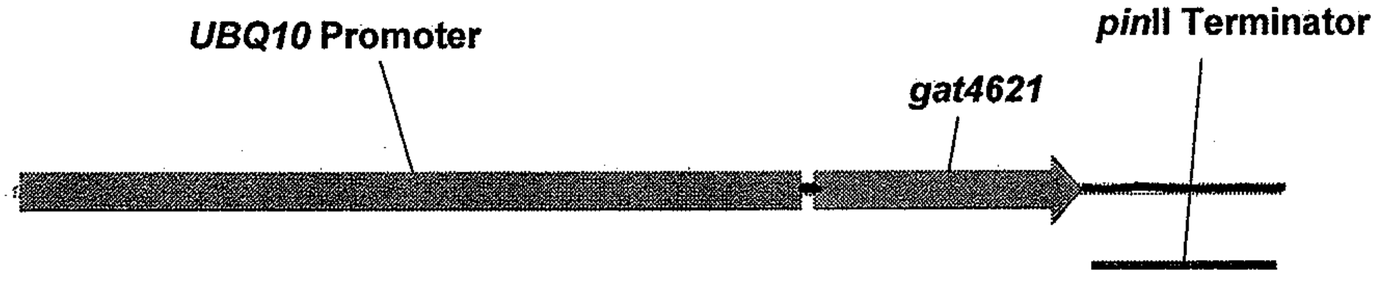


Figure 2: Schematic diagram of plasmid PHP28181



PHP28181A
2112 bp

Figure 3: Schematic Diagram of Fragment PHP28181A

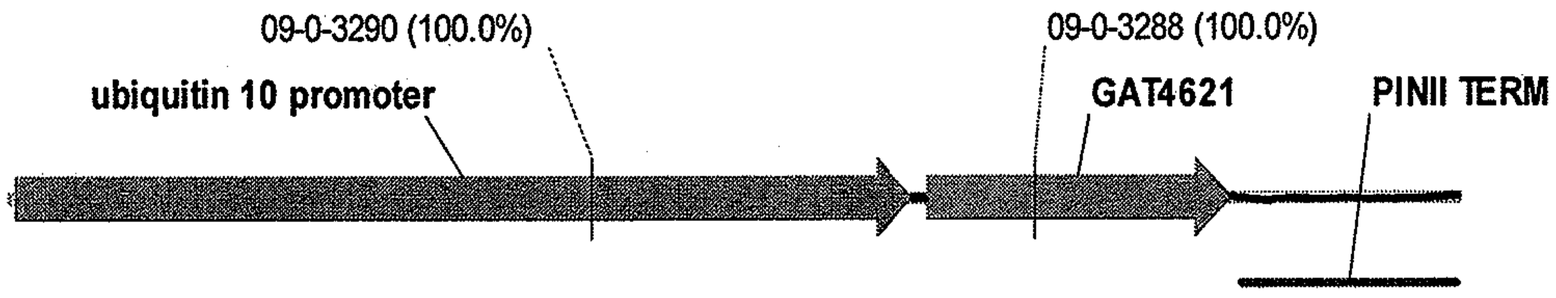


Figure 4: Schematic Representation Of Fragment A From PHP28181 (PHP28181A)

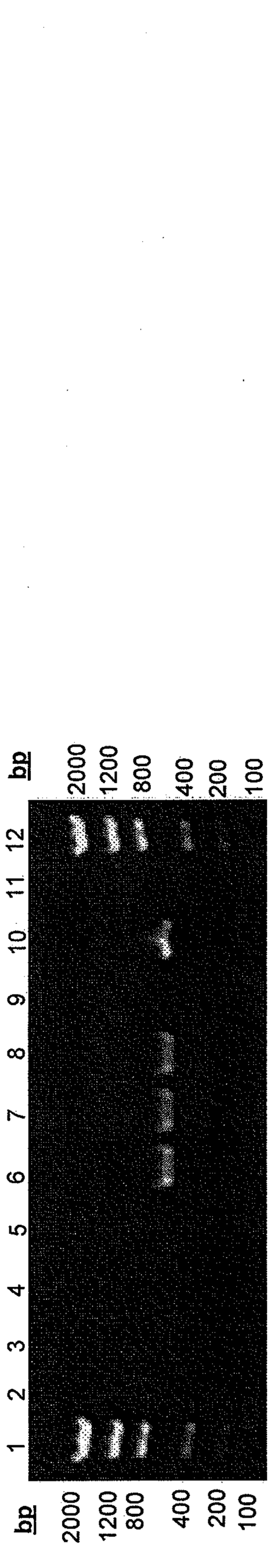


Figure 5: PCR Analysis of Leaf DNA From DP-073486-4 Brassica and Non-Genetically Modified Control Brassica

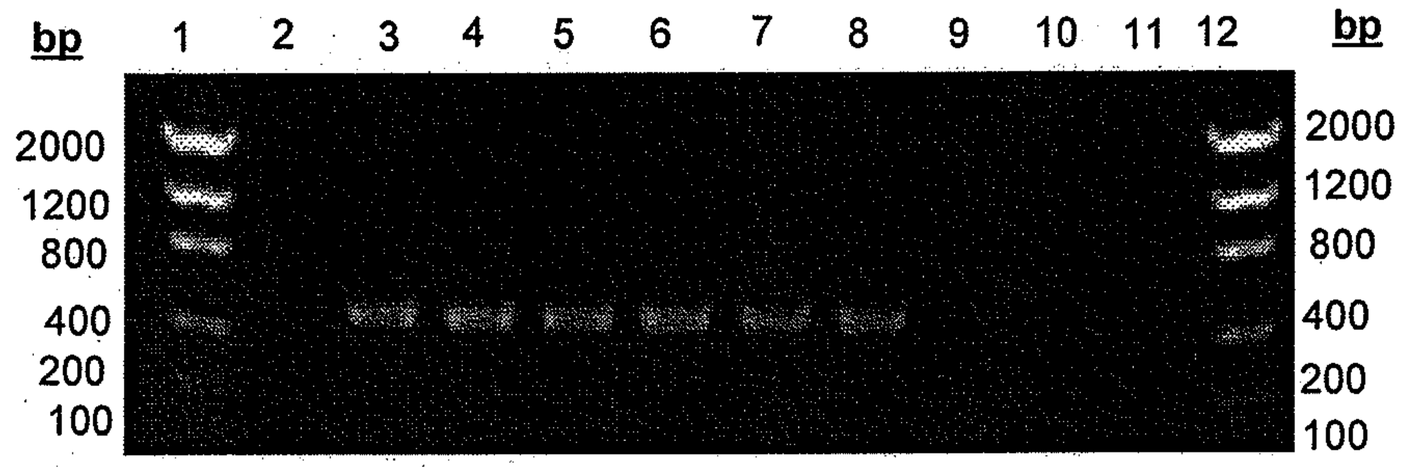


Figure 6: FatA Gene PCR Analysis of Leaf DNA From DP-073496-4 Brassica and Non-Genetically Modified Control Brassica