Study Title

Molecular Analysis of Glyphosate-Tolerant Roundup Ready® 2 (RR2) Canola MON 88302

Authors

Study Completed

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Sponsor/ Testing Facility

Monsanto Company
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Laboratory Project ID

Study REG-09-549
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This study meets the U.S. EPA Good Laboratory Practice requirements as specified in 40 CFR Part 160 with the following exception:

Sequence information used in this study was generated by the Monsanto Genomics Sequencing Center, which does not generate its data in compliance with the GLP regulations.

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Quality Assurance Statement

Study Title: **Molecular Analysis of Glyphosate-Tolerant Roundup Ready® 2 (RR2) Canola MON 88302**

Study Number: REG-09-549

Reviews conducted by the Quality Assurance Unit confirm that the final report accurately describes the methods and standard operating procedures followed and accurately reflects the raw data of the study.

Following is a list of reviews conducted by the Monsanto Regulatory Quality Assurance Unit on the study reported herein.

Dates Of Inspection / Audit	Phase	Date Reported To Study Director	Date Reported To Management
12/08/2009	Southern Blot	12/17/2009	12/17/2009
04/14/2010	Raw Data Audit	04/15/2010	04/15/2010
05/27/2010	Draft Report and Data Audit	05/28/2010	05/28/2010

Quality Assurance Specialist **Date**
Monsanto Regulatory, Monsanto Company

Study Information Page

Study Number(s):	REG-09-549
MSL Number:	MSL0022523
Title:	Molecular Analysis of Glyphosate-Tolerant Roundup Ready® 2 (RR2) Canola MON 88302
Primary Facility:	Monsanto Company Regulatory Product Characterization Center 800 North Lindbergh Blvd. St. Louis, Missouri 63167
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Sponsor Representative:	[redacted]
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Study Director:	[redacted]
Contributors:	[redacted]
Study Initiation Date:	November 19, 2009
Study Completion Date:	May 28, 2010
Records Retention:	All study-specific raw data (including rejected data) protocol, final report and facility records will be retained at Monsanto Company, St. Louis, Missouri.
Sample Storage:	Any study samples that are to be retained will be stored at Monsanto Company, St. Louis, Missouri.

Study Certification Page

This report is an accurate and complete representation of the study/project activities.

[redacted] [redacted]
Regulatory Product Characterization Center

[redacted] **Date**
Molecular Team Lead
Regulatory Product Characterization Center

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Abbreviations[1] and Definitions

~	Approximately
aadA	Bacterial promoter, coding sequence, and 3′ untranslated region for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyl-transferase from the transposon Tn7 that confers spectinomycin and streptomycin resistance
a.e.	Acid equivalent
B-Left Border	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA
B-Right Border	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA
BSA	Bovine Serum Albumin
CP4 EPSPS	5-Enolpyruvylshikimate-3-phosphate synthase protein from the *Agrobacterium* sp. strain CP4
CS-*cp4 epsps*	Codon optimized coding sequence of the *aroA* gene from *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein
CS-*rop*	Coding sequence for repressor of primer protein from the ColE1 plasmid for maintenance of plasmid copy number in *E. coli*
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dNTP	Deoxynucleotide triphosphate
E. coli	*Escherichia coli*
I-*Tsf1*	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 alpha
L-*Tsf1*	5' untranslated leader sequence (exon 1) from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 alpha
OR-*ori-pBR322*	Origin of replication from pBR322 for maintenance of plasmid in *E. coli*
OR-*ori V*	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium*
P-*FMV/Tsf1*	Chimeric promoter consisting of the promoter of the *Tsf1* gene from the *Arabidopsis thaliana* encoding elongation factor EF-1 alpha and enhancer sequences from the 35S promoter from the figwort mosaic virus (FMV)
PCR	Polymerase Chain Reaction
Sarkosyl	*N*-lauroylsarcosine, sodium salt
sp.	Species
T-DNA	Transfer DNA
Tm	Melting temperature

Abbreviations and Definitions (Cont.)

TS-*CTP2*	Sequences encoding the chloroplast transit peptide from the *shkG* gene of *Arabidopsis thaliana* encoding EPSPS
T-*E9*	3′ untranslated region from the *rbcS2* gene of *Pisum sativum* (pea) encoding the Rubisco small subunit

[1]Standard abbreviations, e.g., units of measure, were used in this report according to format described in "Instructions to Authors" in the Journal of Biological Chemistry.

1.0 Summary

Monsanto Company has developed a second generation herbicide-tolerant canola product, MON 88302 that is tolerant to in crop glyphosate application(s) from emergence to first flowering at a rate up to 1800 g a.e. per hectare. With an increased window of application and higher spray rates, MON 88302 will provide superior weed control compared to the commercial first generation Roundup Ready® (RR) canola product RT73 (also referred to as GT73).

MON 88302 was developed through *Agrobacterium*-mediated transformation of conventional canola using plasmid vector PV-BNHT2672. PV-BNHT2672 is comprised of a *cp4 epsps* gene expression cassette, which contains the following genetic elements: *P-FMV/Tsf1* chimeric promoter derived from enhancer sequences of 35S promoter of the figwort mosaic virus (Richins et al., 1987) and the promoter from the *Tsf1* gene of *Arabidopsis thaliana* (Axelos et al., 1989); the leader and intron sequences from the *Arabidopsis thaliana Tsf1* gene; the EPSPS chloroplast transit peptide coding sequence from the *shkG* gene of *Arabidopsis thaliana* (Klee et al., 1987); the codon optimized coding sequence of *cp4 epsps* from *Agrobacterium* sp. strain CP4 (Barry et al., 1997; Padgette et al., 1996); and the polyadenylation sequence derived from the 3' untranslated region of the pea (*Pisum sativum*) ribulose-1,5-bisphosphate carboxylase small subunit (*rbcS2*) *E9* gene (Coruzzi et al., 1984).

In this study, Southern blot analyses were used to confirm the copy number of the integrated DNA sequences and the presence or absence of backbone sequences from the plasmid vector PV-BNHT2672 in the genome of MON 88302. The data show that MON 88302 contains one copy of the T-DNA insert at a single integration locus and all expression elements of the T-DNA are present in the insert. These data also show that MON 88302 does not contain detectable backbone sequences from plasmid vector PV-BNHT2672. The complete DNA sequence of the insert and adjacent genomic DNA sequence in MON 88302 confirmed the integrity of the inserted *cp4 epsps* expression cassette within the inserted T-DNA sequences and identified the 5' and 3' insert-to-genomic DNA junctions. Additional analysis of the insertion site in conventional control Ebony and MON 88302 confirmed that the genomic DNA flanking the DNA insert is native to the canola genome, and showed that a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert and a 29 base pair deletion from the conventional control Ebony genomic DNA occurred during the insertion of the T-DNA into the conventional canola to form MON 88302. Furthermore, Southern blot fingerprint analysis demonstrated that the insert in MON 88302 has been maintained through multiple generations of breeding, thereby confirming the stability of the insert in multiple generations.

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2.0 Introduction

2.1 Background

Monsanto Company has developed a second generation herbicide-tolerant canola product, MON 88302 that is tolerant to in crop glyphosate application(s) from emergence to first flowering at a rate up to 1800 g a.e. per hectare. With an increased window of application and higher spray rates, MON 88302 will provide superior weed control compared to the commercial first generation Roundup Ready® (RR) canola product RT73 (also referred to as GT73). MON 88302 contains the 5-enolpyruvylshikimate-3-phosphate synthase gene derived from *Agrobacterium* sp. strain CP4 (*cp4 epsps*). Expression of the gene product (CP4 EPSPS) renders the plant tolerant to glyphosate, the active ingredient in the Roundup® family of agricultural herbicides.

2.2 Purpose

The purpose of this study was to characterize the integrated DNA in MON 88302. Genomic DNA from MON 88302 was analyzed using Southern blot, PCR, and DNA sequence analysis methodologies. Southern blot analysis was used to determine the number of insertion sites and copies of the integrated T-DNA as well as the presence or absence of plasmid backbone sequences in the genome of MON 88302. Southern blot analyses were also performed on multiple generations of MON 88302 to confirm the stability of the inserted DNA in MON 88302. The complete DNA sequence of the insert and adjacent genomic DNA sequence in MON 88302 was determined by PCR and DNA sequence analyses. In addition, PCR and DNA sequence analyses were performed on the conventional control Ebony genomic DNA to confirm that the genomic DNA sequences flanking the 5' and 3' ends of the insert in MON 88302 are native to the canola genome, thereby determining the integrity and genomic organization of the insertion site in MON 88302.

3.0 Materials and Methods

3.1 Test Substance

The test substance was MON 88302. Genomic DNA extracted from the leaf tissue of the R_3 generation was used in this study.

Test Subtance	Generation	Starting Seed ORION[2] ID	Leaf Tissue ORION ID	Production Plan
MON 88302	R_3	11236103	11238940	PPN-09-307

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[2]ORION is a proprietary database used at Monsanto Company to track plant samples within the Regulatory Organization.

Additional leaf samples from the R_2, R_4, R_{5a} and R_{5b} generations of MON 88302 were used to assess the stability of the T-DNA insert across multiple generations of MON 88302 by Southern blot analysis.

Test Subtance	Generation	Starting Seed ORION ID	Leaf Tissue ORION ID	Production Plan
MON 88302	R_2	11236102	11265247	PPN-09-523
	R_4	11225246	11264678	PPN-09-523
	R_{5a}	11263713	11264681	PPN-09-523
	R_{5b}	11261829	11265248	PPN-09-523

3.2 Control Substance

The control substance was conventional canola Ebony. Genomic DNA extracted from the leaf tissue listed in the table below was used in this study.

Control Substance	Starting Seed ORION ID	Leaf Tissue ORION ID	Production Plan
Conventional Control Ebony	11225244	11238938	PPN-09-307

3.3 Reference Substance

The reference substance was plasmid vector PV-BNHT2672, which was used to develop MON 88302. The identity of the plasmid vector PV-BNHT2672 was confirmed by restriction enzyme digestion. Digested whole plasmid vector and/or probe templates generated from this plasmid vector were used as positive hybridization controls in Southern blot analyses. Additionally, appropriate molecular weight markers from commercial sources were used for size estimations on Southern blots and agarose gels.

3.4 Characterization of Test and Control Substances

The starting seeds for the test and control substances used in this study were obtained from Monsanto Trait Development. The synthesis records for these materials are located in the Virgo, MIDAS, and ORION systems. The Study Director reviewed the chain of custody documentation and confirmed the identity of the test, control, and reference substances prior to use of the materials in the study. No certificates of analysis (COA) or verification of identity (VOI) certificates were generated for these materials. The molecular fingerprint of the test substance was generated in this study, and the identity of the test substance, as well as the absence of the test substance in the conventional control substance, was further confirmed by the methods defined in this study (i.e., Southern blot analyses). The test, control, and reference substances were considered stable during storage if they did not appear degraded on ethidium bromide stained agarose gels and/or if they yielded interpretable signals on Southern blots and/or produced specific PCR products.

3.5 Genomic DNA Isolation

Genomic DNA was isolated from leaf tissue prior to use in the study. The leaf tissue was processed to fine powder in liquid nitrogen prior to extraction. Genomic DNA from the conventional control Ebony and the R_3 generation of MON 88302 was extracted using a modified sarkosyl protocol which was approved by the study director. Genomic DNA from the R_2, R_4, R_{5a} and R_{5b} generations of MON 88302 was extracted using the same sarkosyl method according to SOP AG-ME-1329-01 that came from the study-director-approved protocol. All extracted DNA was stored in a 4°C refrigerator or a -20°C freezer.

3.6 DNA Quantification

Extracted genomic DNA was quantified using a Hoefer DyNA Quant 200 Fluorometer (Hoefer, Inc., Holliston, MA) according to SOP BR-EQ-0065-02.

3.7 Restriction Enzyme Digestion

Approximately ten micrograms (µg) of genomic DNA extracted from MON 88302 and conventional control Ebony were digested with restriction enzyme *Ase* I (New England Biolabs, Inc. Ipswich, MA) and a combination of restriction enzymes *Sal* I and *Sca* I (New England Biolabs, Inc.) according to SOP BR-ME-0316-01. All digests were conducted in 1X NEBuffer 3 (New England Biolabs, Inc.) at 37°C in a total volume of ~500 microliter (µl) with ~50 units of each restriction enzyme. Digests conducted with the combination of restriction enzymes *Sal* I and *Sca* I also included 1X BSA (New England Biolabs, Inc.) in the reaction. For the purpose of running positive hybridization controls, ~10 µg of genomic DNA extracted from the conventional control Ebony was digested with the restriction enzyme *Ase* I and the appropriate positive hybridization control(s) were added to these digests prior to loading the agarose gel.

3.8 Agarose Gel Electrophoresis

Digested DNA was resolved on ~0.8% (w/v) agarose gels according to SOP BR-ME-0315-02. For T-DNA insert/copy number and plasmid vector backbone analyses, individual digests containing ~10 µg each of MON 88302 and conventional control Ebony genomic DNA were loaded on the same gel in a long run/short run format. The long run allows for greater resolution of large molecular weight DNA, whereas the short run allows for the detection of small molecular weight DNA. The positive hybridization controls were only run in the short run format. For the insert stability analysis, individual digests of ~10 µg of genomic DNA extracted from five leaf samples from four generations of MON 88302 and the conventional control Ebony along with the positive hybridization controls were loaded on the agarose gel in a single run format.

3.9 Probe Preparation

Probe templates were prepared by PCR amplification using plasmid vector PV-BNHT2672 as template according to SOP BR-ME-0486-01 and gel purification according to SOP BR-ME-0889-01. The probe templates were designed based on the nucleotide composition (% GC) of the sequence in order to optimize the detection of DNA sequences during hybridization. When possible, probes possessing similar melting temperature (Tm) were combined in the same Southern blot hybridization. Approximately 25 ng of each probe template were radiolabeled with either [α-^{32}P]-deoxycytidine triphosphate (dCTP) (6000 Ci/mmol) or [α-^{32}P]-deoxyadenosine triphosphate (dATP) (6000 Ci/mmol) using RadPrime DNA Labeling System (Invitrogen, Carlsbad, CA) according to SOP BR-ME-0611-01.

3.10 Southern Blot Analyses

Genomic DNA isolated from MON 88302 and the conventional control Ebony was digested and evaluated using Southern blot analyses according to SOP BR-ME-0317-02. Plasmid vector PV-BNHT2672 DNA previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I was added to conventional control Ebony genomic DNA digested with *Ase* I to serve as positive hybridization control on each Southern blot. When multiple probes were hybridized simultaneously to one Southern blot, the probe templates were spiked in the digested conventional control Ebony genomic DNA to serve as additional positive hybridization controls on the Southern blot. The DNA was then separated by agarose gel electrophoresis and transferred onto a nylon membrane. Southern blots were hybridized and washed at 55°C, 60°C, or 65°C, depending on the calculated Tm of the probes that were used. The table below lists the radiolabeling conditions and hybridization temperatures of the probes used in this study. Multiple exposures of each blot were then generated using Kodak Biomax MS film (Eastman Kodak, Rochester, NY) in conjunction with one Kodak Biomax MS intensifying screen in a -80°C freezer.

Probe	DNA Probe	Labeling Method	Probe Labeled with dNTP (^{32}P)	Hybridization/Wash Temperature (°C)
1	T-DNA Probe 1	RadPrime	dATP	55
2	T-DNA Probe 2	RadPrime	dATP	60
3	T-DNA Probe 3	RadPrime	dATP	55
4	Backbone Probe 4	RadPrime	dCTP	65
5	Backbone Probe 5	RadPrime	dCTP	60
6	Backbone Probe 6	RadPrime	dCTP	60

3.11 PCR and DNA Sequence Analyses to Examine the Insert and Flanking Sequences in MON 88302

Overlapping PCR products, denoted as Product A and Product B, were generated that span the insert and adjacent 5' and 3' flanking genomic DNA sequences in MON 88302. These products were sequenced to determine the nucleotide sequence

of the MON 88302 insert as well as the nucleotide sequence of the genomic DNA flanking the 5' and 3' ends of the insert.

The PCR analyses were performed according to SOP BR-ME-0486-01. To generate both Product A and Product B, the PCR reactions were conducted using 30 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 1.5 mM $MgSO_4$ (Novagen, Madison, WI), 0.3 µM of each primer, 0.2 mM of each dNTP (Novagen, Madison, WI), and 2.0 units of KOD Hot Start DNA polymerase (Novagen, Madison, WI). For the generation of Product B, a final concentration of 1 M betaine (USB Corp. Cleveland, OH) was also included in the reaction. The amplification of Product A was performed under the following cycling conditions: 1 cycle at 94°C for 2 minutes; 35 cycles at 94°C for 30 seconds, 65°C for 15 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes. The amplification of Product B was performed under the following cycling conditions: 1 cycle at 94°C for 2 minutes; 35 cycles at 94°C for 30 seconds, 60°C for 15 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium staining to verify that the products were of the expected size. Prior to sequencing, each verified PCR product was purified with the QIAquick PCR Purification Kit (Qiagen, Inc., Valencia, CA) according to manufacturer's instruction and gel quantified according to SOP BR-ME-1222-01. The purified PCR products were sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry (Applied Biosystems, Foster City, CA).

Numerous sequencing reactions performed on the overlapping PCR products were compiled and a consensus sequence was generated. This consensus sequence was aligned with the sequence of plasmid vector PV-BNHT2672 to determine the integrity and genomic organization of the integrated DNA and the junctions of the 5' and 3' flanks in MON 88302.

3.12 PCR and DNA Sequence Analyses to Examine the Integrity of the DNA Insertion Site in MON 88302

To determine the integrity and genomic organization of the insertion site in MON 88302 and to demonstrate that the DNA sequences flanking the insert in MON 88302 are native to the canola genome, PCR analyses were performed on the genomic DNA from both MON 88302 and the conventional control Ebony. The primers used in this analysis were designed from the genomic DNA sequences flanking the insert in MON 88302. A forward primer specific to the genomic DNA sequence flanking the 5' end of the insert was paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert.

The PCR analyses were performed according to SOP BR-ME-0486-01. The PCR reactions were conducted using 30 ng of genomic DNA template in a 50 µl reaction volume containing a final concentration of 2 mM $MgSO_4$, 0.4 µM of each primer, 0.2 mM of each dNTP, and 2.0 units of KOD Hot Start DNA polymerase. The amplification was performed under the following cycling conditions: 1 cycle at 94°C for 2 minutes; 35 cycles at 94°C for 15 seconds, 64°C for 30 seconds, 72°C for 2 minutes; 1 cycle at 72°C for 5 minutes.

Aliquots of each PCR product were separated on 1.0% (w/v) agarose gels and visualized by ethidium staining to verify that a unique PCR product was produced from MON 88302 or the conventional control Ebony genomic DNA. Prior to sequencing, only the verified PCR product from the conventional control Ebony was purified with the QIAquick PCR Purification Kit according to manufacturer's instruction and gel quantified according to SOP BR-ME-1222-01. The purified PCR product was sequenced using multiple primers, including primers used for PCR amplification. All sequencing was performed by the Monsanto Genomics Sequencing Center using BigDye terminator chemistry.

Numerous sequencing reactions performed on the PCR product were compiled and a consensus sequence was generated. This consensus sequence was aligned with the 5' and 3' flanking sequences of the MON 88302 insert to determine the integrity and genomic organization of the insertion site in MON 88302.

3.13 Data Rejected

Some Southern blot analyses conducted as part of this study were rejected. Criteria for rejection included the following: use of incorrect concentration of positive hybridization controls, or background hybridization on the blots. PCR analyses were rejected because the gels were not of reportable quality. None of the rejected data was inconsistent with the conclusions presented in this report.

3.14 Changes to the Study Protocol

During the course of the study, several changes to the original protocol were required, and were documented as a protocol amendment. The changes summarized below had no negative impact on the study.

1. Leaf materials from different generations of MON 88302 for the insert stability analysis were generated after the protocol was signed. The protocol was amended to include those leaf materials and clarify the study procedures used for this Southern blot analysis;
2. The alignment analysis between the MON 88302 DNA sequence and the sequence of the plasmid vector PV-BNHT2672 was added to determine the integrity and genomic organization of the integrated DNA and the junctions of the 5' and 3' flanks in MON 88302;

3. The alignment analyses between the conventional control Ebony sequence and both the 5' and 3' flanking sequences of MON 88302 were added to determine the integrity and genomic organization of the insertion site in MON 88302.

4.0 Results and Discussion

Genomic DNA from MON 88302 was analyzed by Southern blot analyses with probes that spanned the entire plasmid vector, PV-BNHT2672 (Figure 1). These analyses were designed to: 1) determine the insert/copy number of the inserted DNA; 2) determine the presence or absence of plasmid vector PV-BNHT2672 backbone sequences in the genome of MON 88302; 3) demonstrate that the inserted DNA was stably maintained in multiple generations of MON 88302.

Genomic DNA from MON 88302 and the conventional control Ebony was analyzed by PCR and DNA sequence analyses. These analyses were designed to determine: 1) the complete DNA sequence of the insert and adjacent genomic DNA; 2) the organization and intactness of the genetic elements within the insert and the 5′ and 3′ insert-to-genomic DNA junctions of the insert; 3) the organization and integrity of the insertion site in MON 88302 and that the genomic DNA sequences flanking the DNA insert in MON 88302 are native to the canola genome.

4.1 Southern Blot Analyses of MON 88302

Southern blot analysis was used to characterize the DNA insert present in MON 88302. Genomic DNA from MON 88302 was digested with appropriate restriction enzymes and subjected to Southern blot analyses utilizing probes that cover the entire sequence of the plasmid vector PV-BNHT2672. The selection and design of the probes used in this study allowed for the determination of the number of insertion sites and copies of the integrated T-DNA, as well as the presence or absence of the backbone sequences from plasmid vector PV-BNHT2672. Descriptions of the genetic elements and their locations within the plasmid vector PV-BNHT2672 are shown in Table 1. The genetic elements integrated in MON 88302 are summarized in Table 2. A map of the plasmid vector PV-BNHT2672 annotated with the probes used in the Southern analyses is presented in Figure 1. A linear map depicting the restriction sites within the insert DNA sequence, as well as within the known canola genomic DNA immediately flanking the insert in MON 88302 is shown in Figure 2. Based on the linear map of the insert and the plasmid map, a table summarizing the expected DNA fragments for Southern analyses is presented in Table 3. The generations used in this study are depicted in the breeding history diagram shown in Figure 3. The molecular weight markers on the left side of the Southern blot figures (Figure 4 to Figure 8, and Figure 16) were used to estimate the sizes of bands present in the long-run (Figure 4 to Figure 8) or single run (Figure 16) lanes. The molecular weight markers on the right side of the Southern figures (Figure 4 to Figure 8) were used to estimate the sizes of bands present in the short-run lanes. The Southern blots (Figure 4 to Figure 8, and

Figure 16) presented in this report are representative of the data generated in the study.

4.1.1 Southern Blot Analyses to Confirm the Insert and Copy Number of the Inserted T-DNA in MON 88302

The number of copies and insertion sites of T-DNA sequences in the canola genome were evaluated by digesting MON 88302 and conventional control Ebony genomic DNA samples with two sets of restriction enzymes, *Ase* I and the combination of restriction enzymes *Sal* I and *Sca* I. *Ase* I cleaves once within the inserted T-DNA and within the known genomic DNA flanking the 5' and 3' ends of the insert (Figure 2). The combination of restriction enzymes *Sal* I and *Sca* I cleaves once within the inserted T-DNA and within the known genomic DNA flanking the 3' end of the insert (Figure 2). Each set of enzymes generates restriction fragments containing a portion of the T-DNA and adjacent plant genomic DNA, so that each T-DNA insert would generate a unique banding pattern. If T-DNA sequences are present as a single copy at a single integration site in MON 88302, then probing with the sequence from T-DNA should result in the hybridization bands as described in Figure 2 and Table 3. Any additional copies and/or integration sites would be detected as additional bands on the blots.

The Southern blots were hybridized with T-DNA probes that collectively span the entire inserted DNA sequence (Figure 1, Probe 1, Probe 2, and Probe 3). Each Southern blot contains several controls. Conventional control Ebony genomic DNA digested with the restriction enzyme *Ase* I and spiked with either digested plasmid vector PV-BNHT2672 DNA or probe template(s) served as positive hybridization controls. Conventional control Ebony genomic DNA digested with the appropriate restriction enzymes was used as a negative control. The results of these analyses are shown in Figure 4 and Figure 5.

4.1.1.1 Probe 1 and Probe 3

Conventional control Ebony genomic DNA digested with *Ase* I (Figure 4, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 4, Lane 3 and Lane 7) and simultaneously hybridized with Probe 1 and Probe 3 (Figure 1) produced no detectable hybridization bands as expected for the negative control. Conventional control Ebony genomic DNA digested with *Ase* I and spiked with plasmid vector PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure 1), produced two bands at ~7.2 kb and ~2.5 kb (Figure 4, Lane 10), as expected. Conventional control Ebony DNA digested with *Ase* I and spiked with probe templates of Probe 1 and Probe 3 (Figure 1) produced the expected bands at ~2.3 kb and ~1.3 kb (Figure 4, Lane 11 and Lane 12). Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88302 DNA digested with *Ase* I and simultaneously hybridized with Probe 1 and Probe 3 (Figure 1) produced the expected bands at ~3.8 kb and ~1.4 kb (Figure 4, Lane 2 and Lane 6). MON 88302 DNA digested with the combination of restriction enzymes *Sal* I and *Sca* I and hybridized with Probe 1 and Probe 3 (Figure 1) produced two bands at ~2.7 kb and ~4.3 kb (Figure 4, Lane 4 and Lane 8), which is consistent with the expected >1.8 kb and ~4.3 kb bands (Figure 2 and Table 3), respectively.

The results presented in Figure 4 indicate that the sequences covered by Probe 1 and Probe 3 reside at a single detectable locus of integration in MON 88302.

4.1.1.2 Probe 2

Conventional control Ebony DNA digested with *Ase* I (Figure 5, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 5, Lane 3 and Lane 7) and hybridized with Probe 2 (Figure 1) produced no detectable hybridization bands as expected for the negative control. Conventional control Ebony DNA digested with *Ase* I and spiked with plasmid vector PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure 1), produced a unique band at ~2.5 kb (Figure 5, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequences.

MON 88302 DNA digested with *Ase* I and hybridized with Probe 2 (Figure 1) produced the expected band at ~3.8 kb (Figure 5, Lane 2 and Lane 6).
MON 88302 DNA digested with the combination of restriction enzymes *Sal* I and *Sca* I and hybridized with Probe 2 (Figure 1) produced the expected band at ~4.3 kb (Figure 5, Lane 4 and Lane 8).

The results presented in Figure 5 indicate that the sequences covered by Probe 2 reside at a single detectable locus of integration in MON 88302.

4.1.2 Southern Blot Analysis to Determine the Presence or Absence of Backbone Sequences

The presence or absence of the plasmid vector PV-BNHT2672 backbone sequences in MON 88302 genome was evaluated by digesting MON 88302 and conventional control Ebony genomic DNA with two sets of restriction enzymes, *Ase* I and the combination of restriction enzymes *Sal* I and *Sca* I, and hybridizing each blot with one of the three backbone probes that span the entire backbone sequences (Figure 1, Probe 4, Probe 5, and Probe 6). If backbone sequences are present in MON 88302, then probing with backbone probes should result in hybridizing bands. Each Southern blot contains several controls. Conventional control Ebony genomic DNA digested with the restriction enzyme *Ase* I and spiked with digested plasmid vector PV-BNHT2672 DNA served as positive hybridization controls. Conventional control Ebony genomic DNA digested with

the appropriate restriction enzymes was used as a negative control. The results of these analyses are shown in Figure 6, Figure 7, and Figure 8.

4.1.2.1 Probe 4

Conventional control Ebony DNA digested with *Ase* I (Figure 6, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 6, Lane 3 and Lane 7) and hybridized with Probe 4 (Figure 1) produced no detectable hybridization bands as expected for the negative control. Conventional control Ebony DNA digested with *Ase* I and spiked with plasmid vector PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure 1), produced a unique band at ~7.2 kb (Figure 6, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequences.

MON 88302 DNA digested with *Ase* I (Figure 6, Lane 2 and Lane 6) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 6, Lane 4 and Lane 8) and hybridized with Probe 4 produced no detectable bands.

The results presented in Figure 6 indicate that MON 88302 contains no detectable backbone sequences covered by Probe 4.

4.1.2.2 Probe 5

Conventional control Ebony DNA digested with *Ase* I (Figure 7, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 7, Lane 3 and Lane 7) and hybridized with Probe 5 (Figure 1) produced no detectable hybridization bands as expected for the negative control. Conventional control DNA digested with *Ase* I and spiked with plasmid vector PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure 1), produced a unique band at ~7.2 kb (Figure 7, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequences.

MON 88302 DNA digested with *Ase* I (Figure 7, Lane 2 and Lane 6) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 7, Lane 4 and Lane 8) and hybridized with Probe 5 produced no detectable bands.

The results presented in Figure 7 indicate that MON 88302 contains no detectable backbone sequences covered by Probe 5.

4.1.2.3 Probe 6

Conventional control Ebony DNA digested with *Ase* I (Figure 8, Lane 1 and Lane 5) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 8, Lane 3 and Lane 7) and hybridized with Probe 6 (Figure 1) produced no detectable hybridization bands as expected for the negative control. Conventional control Ebony DNA digested with *Ase* I and spiked with plasmid vector PV-BNHT2672

DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure 1), produced a unique band at ~7.2 kb (Figure 8, Lane 10 and Lane 11), as expected. Detection of the positive controls indicates that the probe hybridized to its target sequences.

MON 88302 DNA digested with *Ase* I (Figure 8, Lane 2 and Lane 6) or the combination of restriction enzymes *Sal* I and *Sca* I (Figure 8, Lane 4 and Lane 8) and hybridized with Probe 6 produced no detectable bands.

The results presented in Figure 8 indicate that MON 88302 contains no detectable backbone sequences covered by Probe 6.

4.2 Organization and Sequences of the Insert and Adjacent Genomic DNA in MON 88302

The organization of the elements within the MON 88302 insert was confirmed by PCR amplifying and sequencing two overlapping DNA amplicons that span the entire length of the insert and the associated flanking genomic DNA. The positions of the PCR products relative to the insert, as well as the results of the PCR analyses, are shown in Figure 9.

As expected, no product was obtained with either the control reactions containing no template DNA (Figure 9, Lane 4 and Lane 7) or the conventional control Ebony DNA template (Figure 9, Lane 2 and Lane 5) with any of the primer sets. The reactions containing MON 88302 genomic DNA produced the band at ~3.3 kb for both Product A and Product B (Figure 9, Lane 3 and Lane 6, respectively), as expected. The migration of the molecular weight marker in Lane 1 and the DNA sample in Lane 3 is slightly different when compared to the migration of the molecular weight markers in Lane 8 and the DNA sample in Lane 6. These altered migrations are likely the result of a difference in salt concentrations between the DNA and molecular weight marker preparations (Sambrook and Russell, 2001).

To determine the sequence of the insert in MON 88302 and genomic DNA flanking the insert, the PCR products, Product A (Figure 9, Lane 3) and Product B (Figure 9, Lane 6), were subjected to DNA sequencing analyses. The consensus sequence representing the insert in MON 88302, including the genomic DNA flanking both ends of the insert is shown in Figure 10 and is described in Table 2. This consensus sequence was generated by compiling data from numerous sequencing reactions performed on the overlapping PCR products, Product A and Product B, spanning the length of the insert and genomic DNA flanking the insert.

An alignment between the MON 88302 sequence and the sequence of the plasmid vector PV-BNHT2672 was performed to determine the integrity and genomic organization of the integrated DNA and the junctions of the 5' and 3' flanks in MON 88302. The result showing the consensus sequence from this alignment analysis is shown in Figure 11. This analysis determined that the DNA sequence of the MON 88302 insert is 4428 base pairs (Figure 10, bases 840-5267, and Figure 11),

beginning at base 315 in the Right Border region and ending at base 4742 in the Left Border region of plasmid vector PV-BNHT2672 (Figure 1 and Figure 11). This result demonstrates that the organization of the genetic elements in the insert are consistent with those in plasmid vector PV-BNHT2672 (Figure 11) and are as depicted in Figure 2. From this alignment analysis, a 839 base pair DNA sequence (Figure 10, bases 1-839) immediately flanking the 5' end of the MON 88302 insert, and a 907 base pair DNA sequence (Figure 10, bases 5268-6174) immediately flanking the 3' end of the MON 88302 insert were also determined.

4.3 Organization and Integrity of the DNA Insertion Site in MON 88302

PCR and DNA sequence analyses were performed on genomic DNA extracted from MON 88302 and conventional control Ebony to demonstrate that the DNA sequences flanking the 5' and 3' ends of the insert in MON 88302 are native to the canola genome and to examine the integrity of the DNA insertion site in MON 88302. The PCR was performed with a forward primer specific to the genomic DNA sequence flanking the 5' end of the insert (Figure 12, Primer A) paired with a reverse primer specific to the genomic DNA sequence flanking the 3' end of the insert (Figure 12, Primer B). The results of the PCR analysis are shown in Figure 12.

As expected, no PCR product was generated in the control reaction containing no template DNA (Figure 12, Lane 4). The reaction containing the conventional control Ebony genomic DNA template generated a PCR product at ~1.9 kb (Figure 12, Lane 2). As expected, a ~6.3 kb PCR product (Figure 12, Lane 3) spanning the entire sequence between Primer A and Primer B in MON 88302 was amplified in this analysis.

The ~1.9 kb PCR product generated from the conventional control Ebony genomic DNA was sequenced and the resulting sequence is shown in Figure 13. This consensus sequence was generated by compiling data from numerous sequencing reactions performed on the ~1.9 kb PCR product.

Alignments between the conventional control Ebony sequence and the 5' and 3' flanking sequences of the MON 88302 insert were separately performed to determine the integrity and genomic organization of the insertion site in MON 88302. The result showing the consensus sequence from the alignment between the conventional control Ebony sequence and the 5' flanking sequence of the MON 88302 insert is presented in Figure 14. It was determined by this analysis that base 14 to base 839 of the 5' flanking sequence of the MON 88302 insert are identical to base 1 to base 826 of the conventional control Ebony sequence (Figure 14). The result showing the consensus sequence from the alignment between the conventional control Ebony sequence and the 3' flanking sequence of the MON 88302 insert is presented in Figure 15. It was determined by this analysis that base 5277 to base 6174 of the 3' flanking sequence of the MON 88302 insert are identical to base 856 to base 1753 of the conventional control Ebony sequence, except for one base difference at position 5723 within the 3' flanking sequence of the MON 88302 insert and at

position 1302 of the conventional control Ebony sequence (Figure 15). The difference was most likely caused by a single nucleotide polymorphism segregating in the canola population (Trick et al., 2009). From these alignment analyses, a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert (Figure 10, bases 5268-5276, bold) and a 29 base pair deletion from the conventional Ebony genomic DNA (Figure 13, bases 827-855, bold) were also identified. Those most likely occurred in MON 88302 upon insertion of the T-DNA (Salomon and Puchta, 1998).

This analysis confirms that the DNA sequences flanking the 5' and 3' ends of the insert in MON 88302 are native to the canola genome and that a 9 base pair insertion as well as a 29 base pair deletion occurred during the insertion of the T-DNA into the conventional canola genome to form MON 88302.

4.4 Southern Blot Analysis to Examine Insert Stability in Multiple Generations of MON 88302

In order to demonstrate the stability of the insert in MON 88302, Southern blot analysis was performed using genomic DNA extracted from five samples of leaf tissue from four generations of MON 88302. For reference, the breeding history of MON 88302 is presented in Figure 3. The specific generations tested are indicated in the legend of Figure 3. The R_3 generation was used for the molecular characterization analyses shown in Figure 4 through Figure 15. To analyze insert stability, four samples from another three additional generations of MON 88302 was evaluated by Southern analysis and compared to the R_3 generation. Genomic DNA, isolated from each of the selected generations of MON 88302, was digested with the restriction enzyme *Ase* I (Figure 2) and hybridized with Probe 1 and Probe 3 (Figure 1), which was designed to detect both fragments generated by the *Ase* I digest. Any instability associated with the insert would be detected as extra bands within the fingerprint on the Southern blot. The Southern blot has the same controls as described in Section 4.1.1.1. The results are shown in Figure 16.

Conventional control Ebony genomic DNA digested with restriction enzyme *Ase* I and simultaneously hybridized with Probe 1 and Probe 3 produced no hybridization signals (Figure 16, Lane 1) as expected for the negative control. Conventional control Ebony DNA digested with *Ase* I and spiked with plasmid vector PV-BNHT2672 DNA, previously digested with the combination of restriction enzymes *Bam* HI and *Sca* I (Figure 1 and Table 3), produced the expected bands at ~2.5 kb and ~7.2 kb (Figure 16, Lane 8). Conventional control Ebony DNA digested with *Ase* I and spiked with probe templates of Probe 1 and Probe 3 produced the expected bands at ~2.3 kb and ~1.3 kb (Figure 16, Lane 9 and Lane 10). Detection of the positive controls indicates that the probes hybridized to their target sequences.

MON 88302 genomic DNA digested with *Ase* I and hybridized with Probe 1 and Probe 3 (Figure 1) is expected to produce a Southern fingerprint with two bands at ~3.8 kb and ~1.4 kb (Figure 2 and Table 3). Southern fingerprints produced from the

additional generations, R_2, R_4, R_{5a}, and R_{5b} (Figure 16, Lane 2, Lane 4, Lane 5, and Lane 6), of MON 88302 is consistent with the one produced from the fully characterized generation R_3 (Figure 4, Lane 2 and Lane 6, and Figure 16, Lane 3), indicating that MON 88302 contains one copy of the T-DNA insert that is stable across multiple generations.

5.0 Conclusions

Molecular characterization of MON 88302 by Southern blot analyses demonstrated that the T-DNA was inserted into the canola genome at a single locus containing one copy of the *cp4 epsps* expression cassette. No additional elements were detected other than those associated with the reported insert. Moreover, no plasmid backbone sequences were detected in the genome of MON 88302.

PCR and DNA sequence analyses performed on MON 88302 determined the complete DNA sequence of the insert and adjacent genomic DNA sequences in MON 88302, confirmed the predicted organization of the genetic elements within the insert, determined the 5' and 3' insert-to-genomic DNA junctions, and confirmed that the genomic DNA sequences flanking the 5' and 3' ends of the insert in MON 88302 are native to the canola genome. It was also determined that a 9 base pair insertion immediately adjacent to the 3' end of the MON 88302 insert and a 29 base pair deletion from the conventional Ebony genomic DNA occurred during the insertion of the T-DNA into the conventional canola to form MON 88302.

Southern blot analysis of multiple MON 88302 generations demonstrated that the inserted DNA has been stably maintained through multiple generations of breeding, thereby, confirming the stability of the insert.

6.0 References

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Table 1. Summary of Genetic Elements in Plasmid Vector PV-BNHT2672

Genetic Element	Location in Plasmid	Function (Reference)
T-DNA		
B[1]-Right Border	1-357	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Intervening Sequence	358-427	Sequence used in DNA cloning
P[2]-*FMV/Tsf1*	428-1467	Chimeric promoter consisting of the promoter of the *Tsf1* gene from the *Arabidopsis thaliana* encoding elongation factor EF-1alpha (Axelos et al., 1989) and enhancer sequences from the 35S promoter from the figwort mosaic virus (Richins et al., 1987)
L[3]-*Tsf1*	1468-1513	5' untranslated leader (exon 1) from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 alpha (Axelos et al., 1989)
I[4]-*Tsf1*	1514-2135	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1alpha (Axelos et al., 1989)
Intervening Sequence	2136-2144	Sequence used in DNA cloning
TS[5]-*CTP2*	2145-2372	Sequences encoding the chloroplast transit peptide from the *shkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee et al., 1987)
CS[6]-*cp4 epsps*	2373-3740	Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996)
Intervening Sequence	3741-3782	Sequence used in DNA cloning
T[7]-*E9*	3783-4425	3' untranslated sequence from the *rbcS2* gene of *Pisum sativum* (pea) encoding the Rubisco small subunit (Coruzzi et al., 1984)
Intervening Sequence	4426-4468	Sequence used in DNA cloning
B-Left Border	4469-4910	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)

Table 1. Summary of Genetic Elements in Plasmid Vector PV-BNHT2672 (Cont.)

Genetic Element	Location in Plasmid	Function (Reference)
Vector Backbone		
Intervening Sequence	4911-4996	Sequence used in DNA cloning
OR[8]- *ori V*	4997-5393	Origin of replication from the broad host range plasmid RK2 for maintenance of plasmid in *Agrobacterium* (Stalker et al., 1981)
Intervening Sequence	5394-6901	Sequence used in DNA cloning
CS-*rop*	6902-7093	Coding sequence for repressor of primer protein for maintenance of plasmid copy number in *E. coli* (Giza and Huang, 1989)
Intervening Sequence	7094-7520	Sequence used in DNA cloning
OR-*ori-pBR322*	7521-8109	Origin of replication from pBR322 for maintenance of plasmid in *E. coli* (Sutcliffe, 1979)
Intervening Sequence	8110-8639	Sequence used in DNA cloning
aadA	8640-9528	Bacterial promoter, coding sequence, and 3′ untranslated region for an aminoglycoside-modifying enzyme, 3"(9)-*O*-nucleotidyl-transferase from the transposon Tn7 (Fling et al., 1985) that confers spectinomycin and streptomycin resistance
Intervening Sequence	9529-9664	Sequence used in DNA cloning

[1] B, Border
[2] P, Promoter
[3] L, Leader
[4] I, Intron
[5] TS, Targeting Sequence
[6] CS, Coding Sequence
[7] T, Transcription Termination Sequence
[8] OR, Origin of Replication

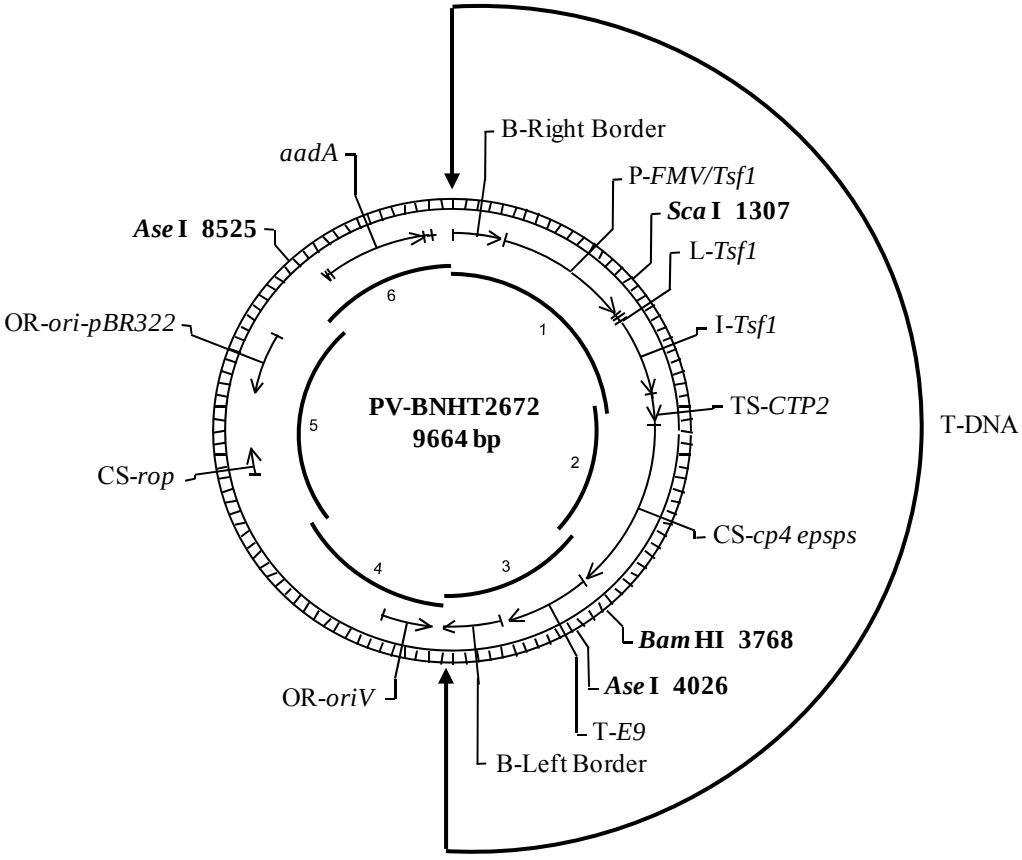
Table 2. Summary of Genetic Elements in MON 88302

Genetic Element[1]	**Location in Sequence**[2]	**Function (Reference)**
Sequence Flanking 5' End of the Insert	1-839	Canola genomic DNA
B[3]**-Right Border** [r1]	840-882	DNA region from *Agrobacterium tumefaciens* containing the right border sequence used for transfer of the T-DNA (Depicker et al., 1982; Zambryski et al., 1982)
Intervening Sequence	883-952	Sequence used in DNA cloning
P[4]***-FMV/Tsf1***	953-1992	Chimeric promoter consisting of the promoter of the *Tsf1* gene from the *Arabidopsis thaliana* encoding elongation factor EF-1alpha (Axelos et al., 1989) and enhancer sequences from the 35S promoter from the figwort mosaic virus (Richins et al., 1987)
L[5]***-Tsf1***	1993-2038	5' untranslated leader (exon 1) from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1 alpha (Axelos et al., 1989)
I[6]***-Tsf1***	2039-2660	Intron from the *Arabidopsis thaliana Tsf1* gene encoding elongation factor EF-1alpha (Axelos et al., 1989)
Intervening Sequence	2661-2669	Sequence used in DNA cloning
TS[7]***-CTP2***	2670-2897	Sequences encoding the chloroplast transit peptide from the *shkG* gene of *Arabidopsis thaliana* encoding EPSPS (Klee et al., 1987)
CS[8]***-cp4 epsps***	2898-4265	Codon optimized coding sequence of the *aroA* gene from the *Agrobacterium* sp. strain CP4 encoding the CP4 EPSPS protein (Barry et al., 1997; Padgette et al., 1996)
Intervening Sequence	4266-4307	Sequence used in DNA cloning
T[9]***-E9***	4308-4950	3' nontranslated sequence from the *rbcS2* gene of *Pisum sativum* encoding the Rubisco small subunit (Coruzzi et al., 1984)

Table 2. Summary of Genetic Elements in MON 88302 (Cont.)

Intervening Sequence	4951-4993	Sequence used in DNA cloning
B-Left Border [r1]	4994-5267	DNA region from *Agrobacterium tumefaciens* containing the left border sequence used for transfer of the T-DNA (Barker et al., 1983)
Sequence Flanking 3' End of the Insert	5268-6174	Canola genomic DNA and 9 bp re-arranged DNA at the site of insertion

[1] Although flanking sequences and intervening sequences are not functional genetic elements, they comprise a portion of the sequence reported in Figure 10.
[2] Numbering refers to the sequence from Figure 10 that includes the insert in MON 88302 and adjacent genomic DNA.
[3] B, Border
[4] P, Promoter
[5] L, Leader
[6] I, Intron
[7] TS, Targeting Sequence
[8] CS, Coding Sequence
[9] T, Transcription Termination Sequence
[r1] Superscripts in Left and Right Borders indicate that the sequences in MON 88302 were truncated compared to the sequences in the plasmid vector PV-BNHT2672.



Probe	DNA Probe	Start Position (bp)	End Position (bp)	Total Length (kb)
1	T-DNA Probe 1	1	2287	~2.3
2	T-DNA Probe 2	2231	3618	~1.4
3	T-DNA Probe 3	3562	4910	~1.3
4	Backbone Probe 4	4911	6564	~1.7
5	Backbone Probe 5	6512	8383	~1.9
6	Backbone Probe 6	8329	9664	~1.3

Figure 1. Map of Plasmid Vector PV-BNHT2672 Showing Probe 1 through Probe 6

A circular map of the plasmid vector PV-BNHT2672 used to develop MON 88302 is shown. Genetic elements and restriction sites used in Southern analyses (with positions relative to the size of the plasmid vector) are shown on the exterior of the map. The probes used in the Southern analyses are shown on the interior of the map and listed in the table. PV-BNHT2672 contains a single T-DNA.

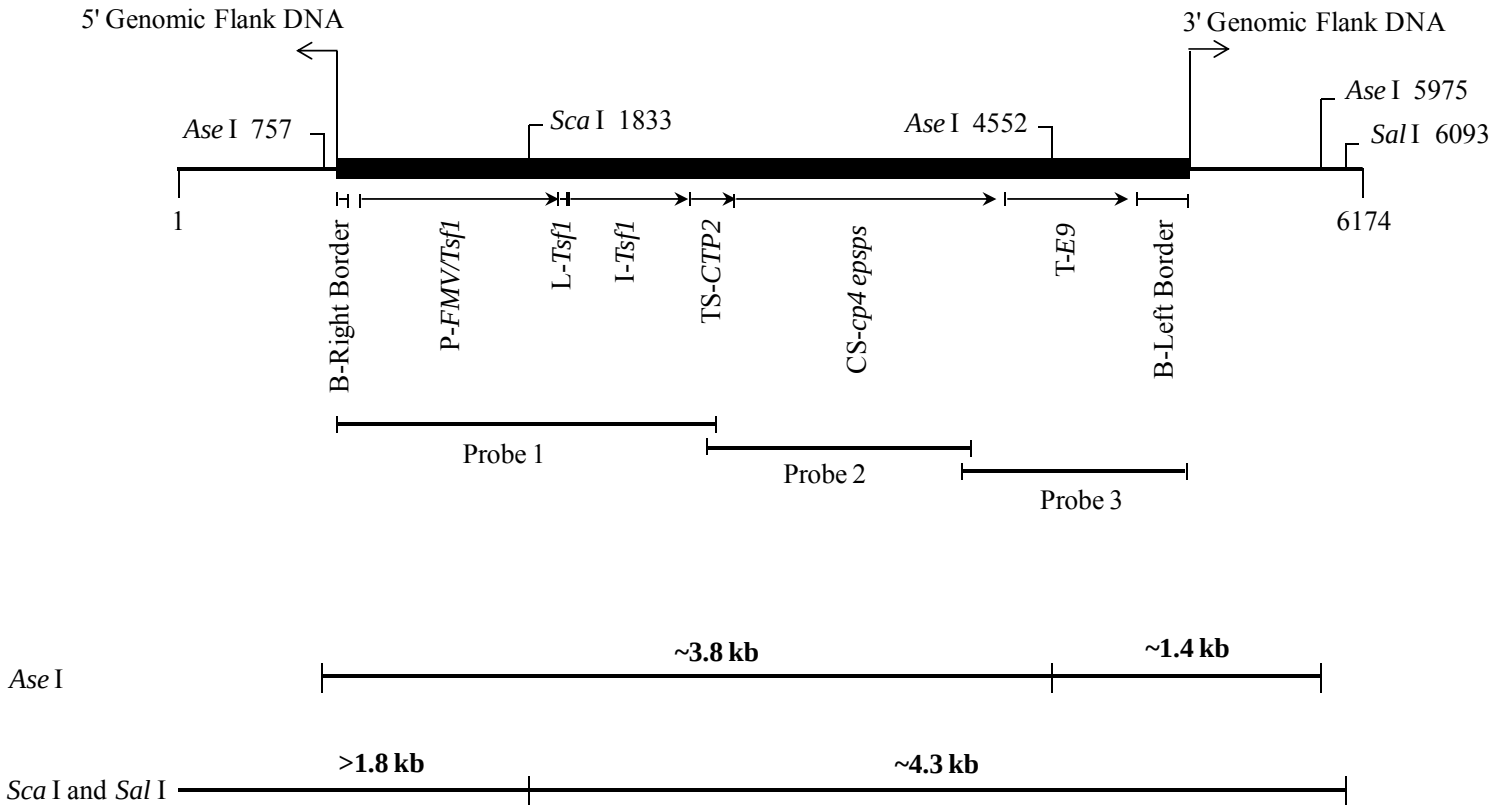


Figure 2. Schematic Representation of the Insert and Genomic Flanking Sequences in MON 88302

A linear map of the insert and genomic DNA flanking the insert in MON 88302 is shown. Right-angled arrows indicate the ends of the integrated T-DNA and the beginning of the flanking sequences. Identified on the linear map are genetic elements within the insert, as well as the sites of the restriction enzymes used in the Southern analyses with positions relative to the size of the DNA sequence (genomic flanks and insert). The relative sizes and locations of the T-DNA probes and the expected sizes of restriction fragments are indicated. This schematic diagram is not drawn to scale. Locations of genetic elements, restriction sites, and T-DNA probes are approximate. Probes are described in Figure 1.

Table 3. Summary Chart of the Expected DNA Fragments Based on Restriction Enzymes and Probes

Southern Blot Analysis		**T-DNA**		**Backbone**			**Insert Stability**
Figure Number		4	5	6	7	8	16
Probe Used		1, 3	2	4	5	6	1, 3
Probing Target	**Digestion Enzyme**	**Expected Band Sizes on Each Southern Blot**					
Plasmid PV-BNHT2672	*Bam* HI and *Sca* I	~2.5 kb ~7.2 kb	~2.5 kb	~7.2 kb	~7.2 kb	~7.2 kb	~2.5 kb ~7.2 kb
Probe Template Spikes[1]	N/A	~2.3 kb ~1.3 kb	~~[2]	~~[2]	~~[2]	~~[2]	~2.3 kb ~1.3 kb
MON 88302	*Ase* I	~3.8 kb ~1.4 kb	~3.8 kb	No band	No band	No band	~3.8 kb ~1.4 kb
	Sal I and *Sca* I	>1.8 kb ~4.3 kb	~4.3 kb	No band	No band	No band	--[3]

[1] probe template spikes were used as positive hybridization controls in Southern blot analyses when multiple probes were hybridized to the Southern blot simultaneously.
[2] '~~' indicates that probe template spikes were not used.
[3] '--' indicates that the combination of the restriction enzymes was not used in the analysis.

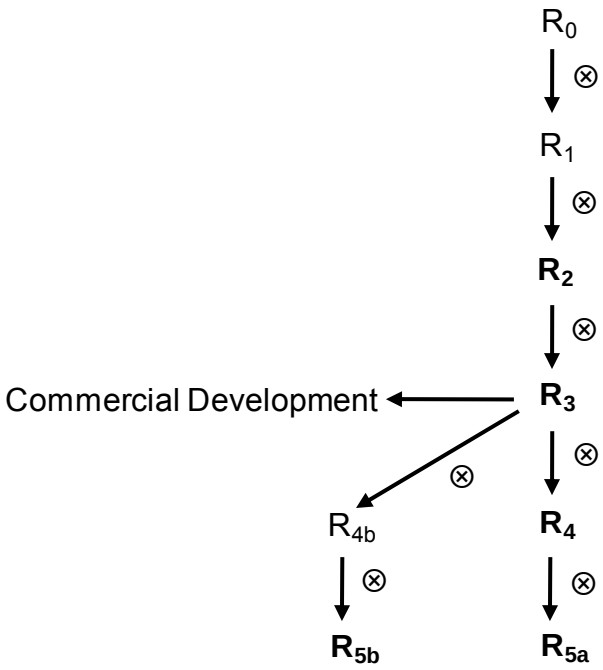


Figure 3. MON 88302 Breeding History Diagram

R_0 corresponds to the transformed canola plant. All generations were self pollinated. ⊗ designates self-pollination. The R_3 generation was used for the molecular characterization and commercial development of MON 88302. The R_2, R_3, R_4, R_{5a}, and R_{5b} (bolded in the breeding tree) generations of MON 88302 were used for analyzing the stability of the insert across generations.

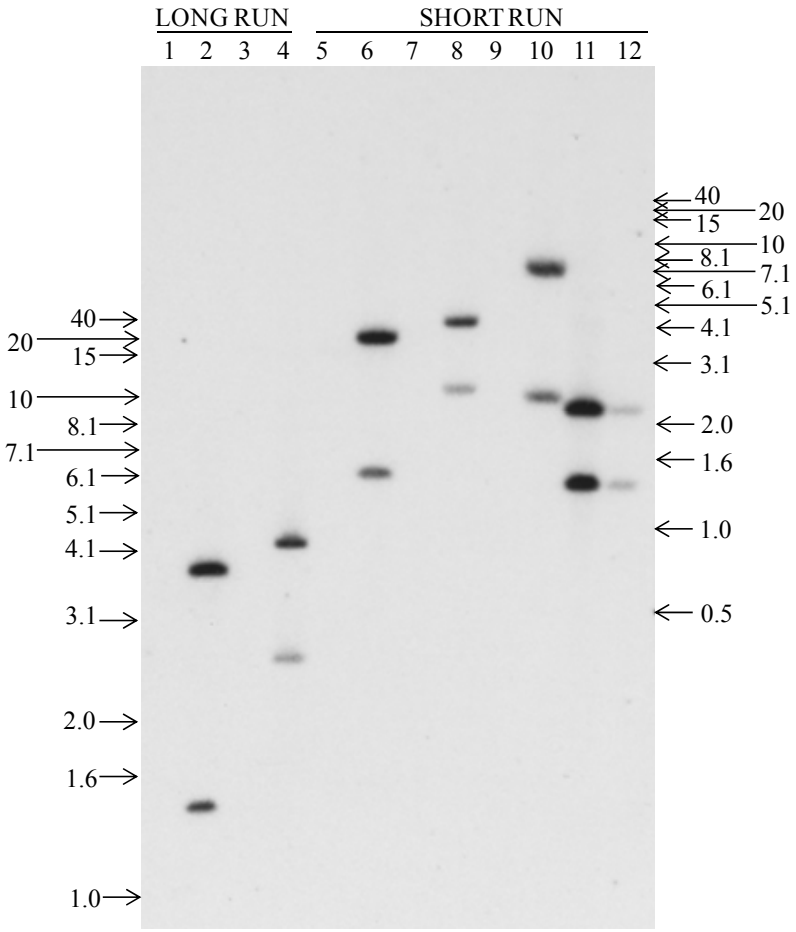


Figure 4. Southern Blot Analysis of MON 88302: T-DNA (Probe 1 and Probe 3)

The blot was simultaneously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure 1, Probe 1 and Probe 3). Each lane contains approximately 10 µg of digested genomic DNA. Lane designations are as follows:

Lane

1 Conventional control Ebony (*Ase* I)
2 MON 88302 (*Ase* I)
3 Conventional control Ebony (*Sal* I/*Sca* I)
4 MON 88302 (*Sal* I/*Sca* I)
5 Conventional control Ebony (*Ase* I)
6 MON 88302 (*Ase* I)
7 Conventional control Ebony (*Sal* I/*Sca* I)
8 MON 88302 (*Sal* I/*Sca* I)
9 Blank
10 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11 Conventional control Ebony (*Ase* I) spiked with Probe 1 and Probe 3 [~1 genome equivalent]
12 Conventional control Ebony (*Ase* I) spiked with Probe 1 and Probe 3 [~0.1 genome equivalent]

Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

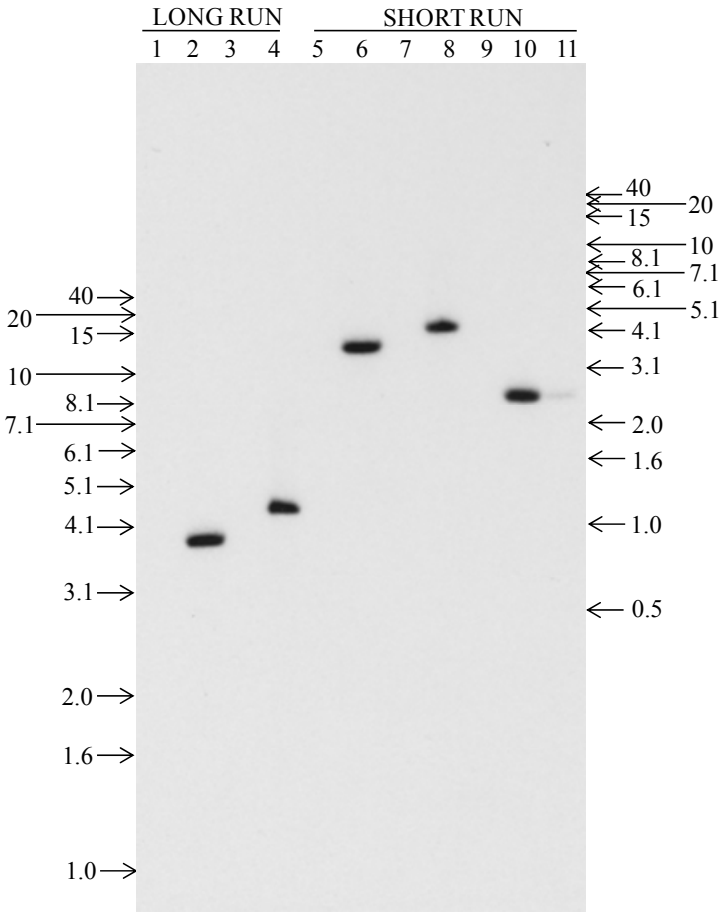


Figure 5. Southern Blot Analysis of MON 88302: T-DNA (Probe 2)

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the T-DNA sequence (Figure 1, Probe 2). Each lane contains approximately 10 µg of digested genomic DNA. Lane designations are as follows:

Lane

1 Conventional control Ebony (*Ase* I)
2 MON 88302 (*Ase* I)
3 Conventional control Ebony (*Sal* I/*Sca* I)
4 MON 88302 (*Sal* I/*Sca* I)
5 Conventional control Ebony (*Ase* I)
6 MON 88302 (*Ase* I)
7 Conventional control Ebony (*Sal* I/*Sca* I)
8 MON 88302 (*Sal* I/*Sca* I)
9 Blank
10 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

Arrows denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

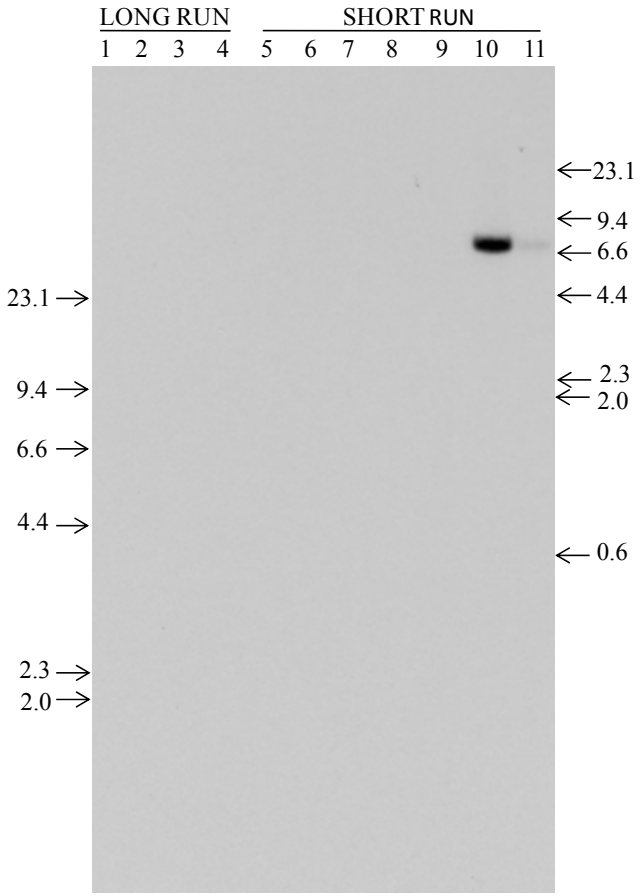


Figure 6. Southern Blot Analysis of MON 88302: PV-BNHT2672 Backbone Sequence Analysis (Probe 4)

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the plasmid vector backbone sequence (Figure 1, Probe 4). Each lane contains approximately 10 µg of digested genomic DNA. Lane designations are as follows:

Lane

1 Conventional control Ebony (*Ase* I)
2 MON 88302 (*Ase* I)
3 Conventional control Ebony (*Sal* I/*Sca* I)
4 MON 88302 (*Sal* I/*Sca* I)
5 Conventional control Ebony (*Ase* I)
6 MON 88302 (*Ase* I)
7 Conventional control Ebony (*Sal* I/*Sca* I)
8 MON 88302 (*Sal* I/*Sca* I)
9 Blank
10 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III Fragments (Invitrogen) on the ethidium bromide stained gel.

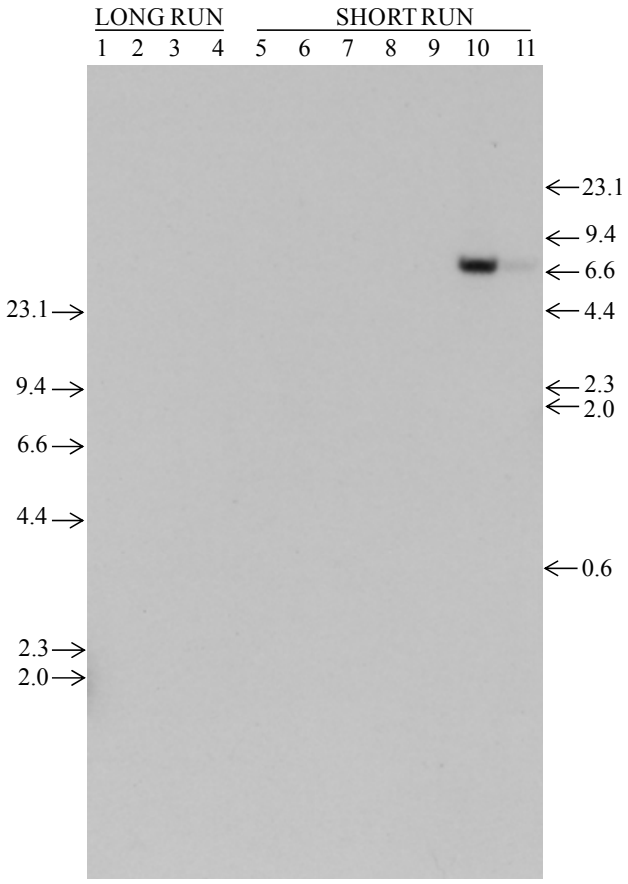


Figure 7. Southern Blot Analysis of MON 88302: PV-BNHT2672 Backbone Sequence Analysis (Probe 5)

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the plasmid vector backbone sequence (Figure 1, Probe 5). Each lane contains approximately 10 µg of digested genomic DNA. Lane designations are as follows:

Lane

1 Conventional control Ebony (*Ase* I)
2 MON 88302 (*Ase* I)
3 Conventional control Ebony (*Sal* I/*Sca* I)
4 MON 88302 (*Sal* I/*Sca* I)
5 Conventional control Ebony (*Ase* I)
6 MON 88302 (*Ase* I)
7 Conventional control Ebony (*Sal* I/*Sca* I)
8 MON 88302 (*Sal* I/*Sca* I)
9 Blank
10 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III Fragments (Invitrogen) on the ethidium bromide stained gel.

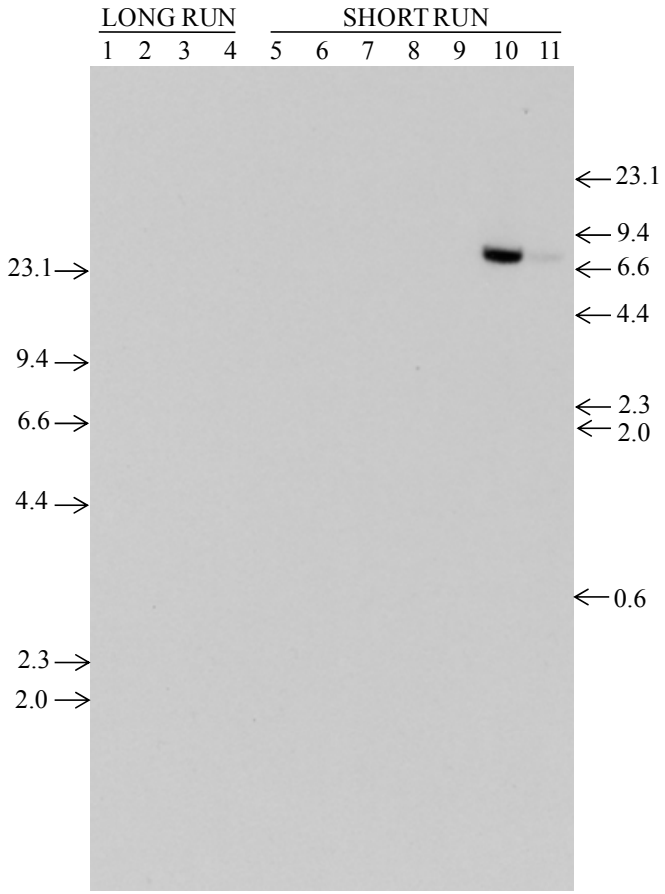


Figure 8. Southern Blot Analysis of MON 88302: PV-BNHT2672 Backbone Sequence Analysis (Probe 6)

The blot was hybridized with a ^{32}P-labeled probe that spans a portion of the plasmid vector backbone sequence (Figure 1, Probe 6). Each lane contains approximately 10 µg of digested genomic DNA. Lane designations are as follows:

Lane

1 Conventional control Ebony (*Ase* I)
2 MON 88302 (*Ase* I)
3 Conventional control Ebony (*Sal* I/*Sca* I)
4 MON 88302 (*Sal* I/*Sca* I)
5 Conventional control Ebony (*Ase* I)
6 MON 88302 (*Ase* I)
7 Conventional control Ebony (*Sal* I/*Sca* I)
8 MON 88302 (*Sal* I/*Sca* I)
9 Blank
10 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
11 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~0.1 genome equivalent]

Arrows denote the size of the DNA, in kilobase pairs, obtained from λ DNA/*Hind* III Fragments (Invitrogen) on the ethidium bromide stained gel.

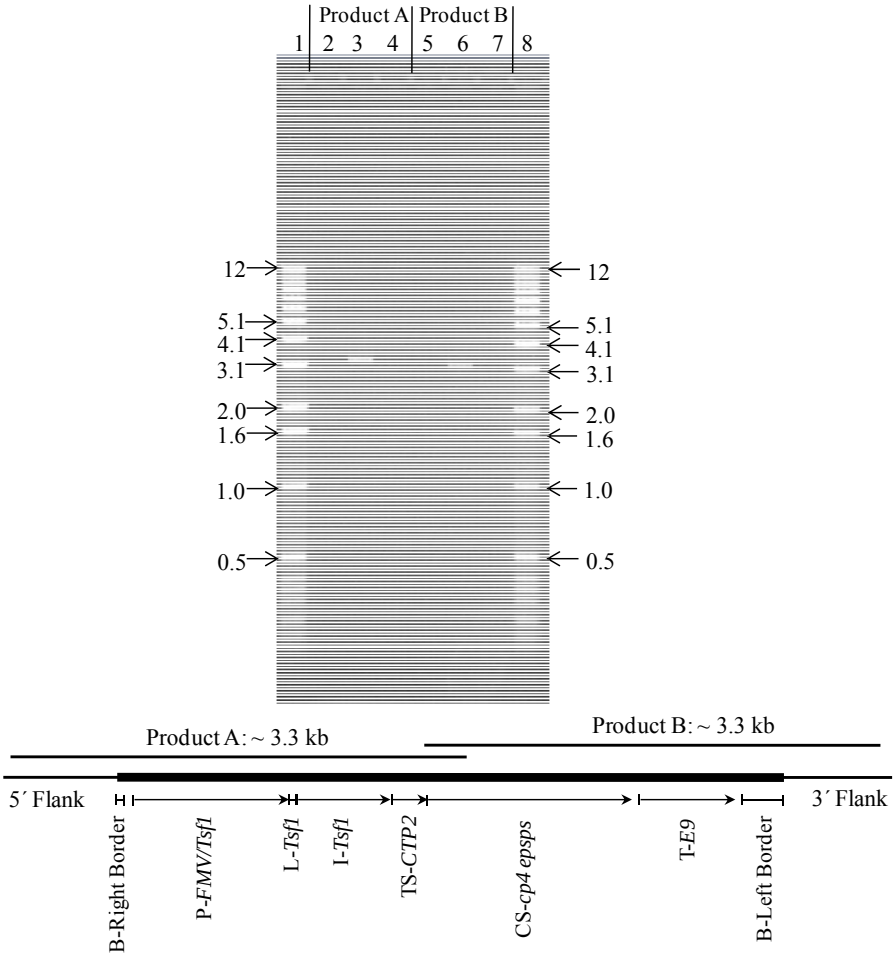


Figure 9. Overlapping PCR Analysis Across the Insert in MON 88302

PCR was performed on both conventional control Ebony genomic DNA and MON 88302 genomic DNA using two pairs of primers to generate overlapping PCR fragments from MON 88302 for sequencing analysis. To verify the PCR products, 5 µl of each of the PCR reactions was loaded on the gel. The expected product size for each amplicon is provided in the illustration of the insert in MON 88302 that appears at the bottom of the figure. This figure is a representative of the data generated in the study. Lane designations are as follows:

Lane

1 1 Kb DNA Ladder
2 Conventional control Ebony
3 MON 88302
4 No template DNA control
5 Conventional control Ebony
6 MON 88302
7 No template DNA control
8 1 Kb DNA Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from 1 Kb DNA Ladder (Invitrogen) on the ethidium bromide stained gel.

[CBI CROSS REFERENCE 1]

[CBI CROSS REFERENCE 2]

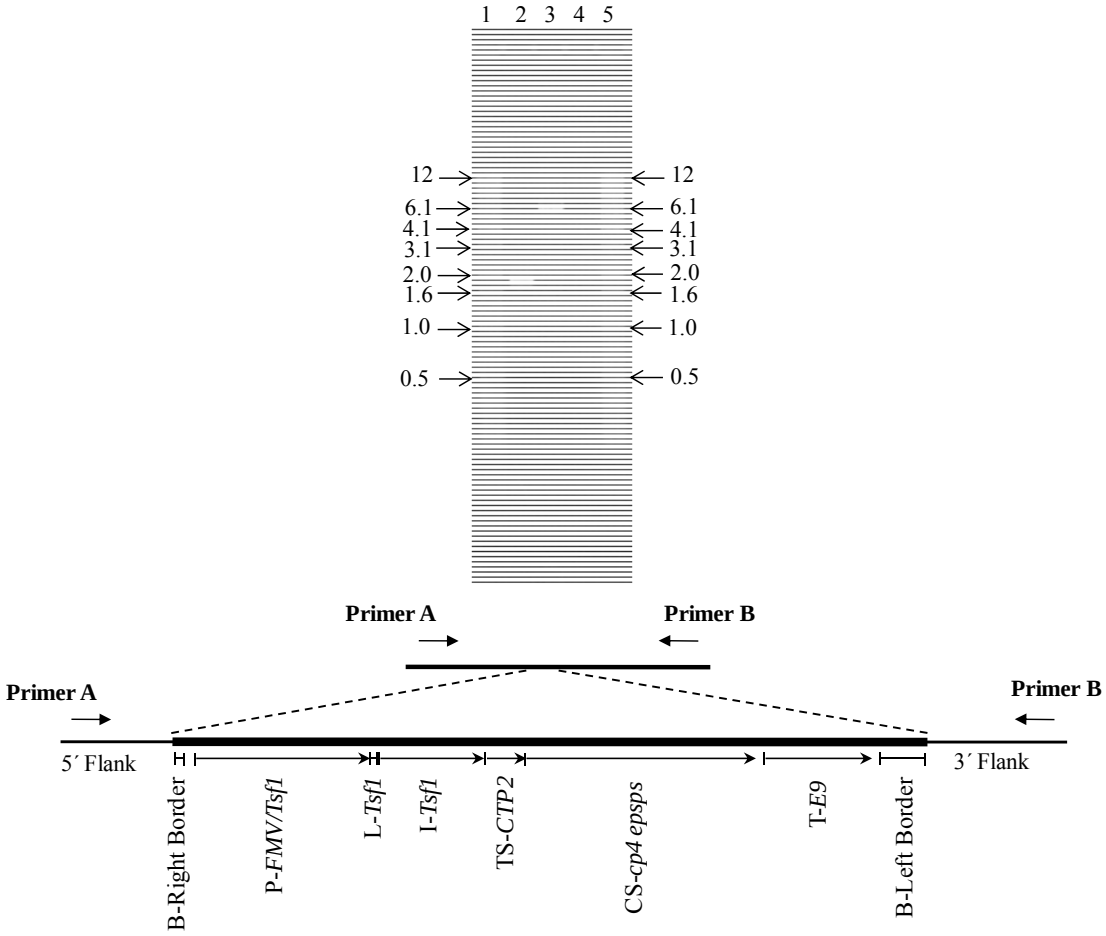


Figure 12. PCR Amplification of the MON 88302 Insertion Site in Conventional Control Ebony and MON 88302

PCR was performed on both conventional control Ebony genomic DNA and MON 88302 genomic DNA, using Primer A specific to the 5' flanking sequence and Primer B specific to the 3' flanking sequence of the insert in MON 88302, to generate DNA fragment from conventional control Ebony for sequencing analysis. The MON 88302 insertion site in conventional control Ebony and the MON 88302 insert are depicted by the upper panel and lower panel, respectively. To verify the PCR products, 5 µl of each of the PCR reactions were loaded on the gel. This figure is a representative of the data generated in the study. Lane designations are as follows:

Lane

1 1 Kb DNA Ladder
2 Conventional control Ebony
3 MON 88302
4 No template DNA control
5 1 Kb DNA Ladder

Arrows on the agarose gel photograph denote the size of the DNA, in kilobase pairs, obtained from 1Kb DNA Ladder (Invitrogen) on the ethidium bromide stained gel.

[CBI CROSS REFERENCE 3]

[CBI CROSS REFERENCE 4]

[CBI CROSS REFERENCE 5]

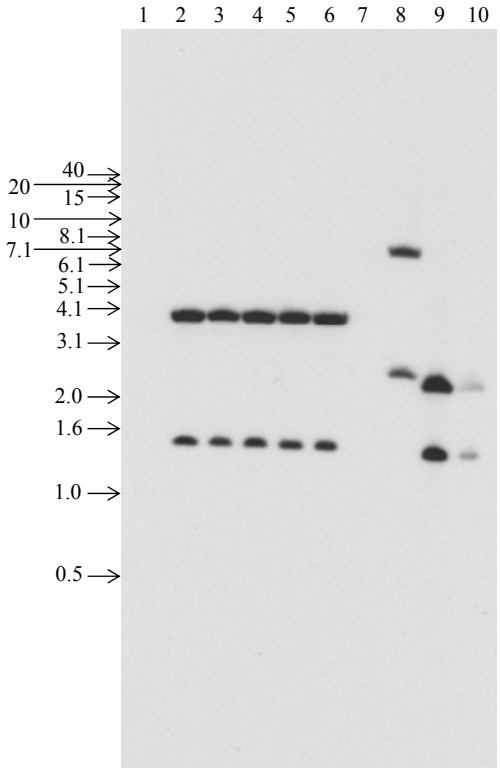


Figure 16. Insert Stability of MON 88302: T-DNA (Probe 1 and Probe 3)

The blot was simultinously hybridized with two ^{32}P-labeled probes that span a portion of the T-DNA sequence (Figure 1, Probe 1 and Probe 3). Each lane contains ~10 µg of digested genomic DNA. Lane designations are as follows:

Lane

1 Conventional control Ebony (*Ase* I)
2 R_2 generation of MON 88302 (*Ase* I)
3 R_3 generation of MON 88302 (*Ase* I)
4 R_4 generation of MON 88302 (*Ase* I)
5 R_{5a} generation of MON 88302 (*Ase* I)
6 R_{5b} generation of MON 88302 (*Ase* I)
7 Blank
8 Conventional control Ebony (*Ase* I) spiked with PV-BNHT2672 (*Bam* HI/*Sca* I) [~1 genome equivalent]
9 Conventional control Ebony (*Ase* I) spiked with probe templates Probe 1 and Probe 3 [~1 genome equivalent]
10 Conventional control Ebony (*Ase* I) spiked with probe templates Probe 1 and Probe 3 [~0.1 genome equivalent]

Arrows denote the size of the DNA, in kilobase pairs, obtained from 1Kb DNA Extension Ladder (Invitrogen) on the ethidium bromide stained gel.

Appendix 1. Standard Operating Procedures

AG-ME-1329-01	Modified Sarkosyl Method for DNA Extraction from Leaf Tissue
BR-EQ-0065-02	DyNA Quant 200 Fluorometer
BR-ME-0315-02	Agarose Gel Electrophoresis
BR-ME-0316-01	Restriction Enzyme Digestion of DNA
BR-ME-0317-02	Southern Blot Analysis
BR-ME-0486-01	Polymerase Chain Reaction
BR-ME-0611-01	Radiolabeling of Nucleic Acids
BR-ME-0889-01	Purification of DNA from an Agarose Gel (Adaptation of Qiagen Gel Extraction Kit)
BR-ME-1222-01	DNA Quantification Using Invitrogen High and Low DNA Mass Ladders

CONFIDENTIAL ATTACHMENT

Study Title

Molecular Analysis of Glyphosate-Tolerant Roundup Ready® 2 (RR2) Canola MON 88302

Authors

[redacted]

Study Completed

May 28, 2010

Sponsor/ Testing Facility

Monsanto Company
Regulatory Product Characterization Center
800 North Lindbergh Blvd.
St. Louis, MO 63167

Laboratory Project ID

Study REG-09-549
MSL0022523

CONFIDENTIAL ATTACHMENT

CBI CROSS REFERENCE 1

Deleted Pages: Figure 10. DNA Sequence of the Insert and Adjacent Genomic DNA in MON 88302

Pages	Reason for Deletion	FIFRA Reference
43-44	Discloses Manufacturing or Quality Control Processes	10(d)

CBI CROSS REFERENCE 2

Deleted Pages: Figure 11. Alignment of the MON 88302 Sequence with the Sequence of Plasmid Vector PV-BNHT2672

Pages	Reason for Deletion	FIFRA Reference
45-50	Discloses Manufacturing or Quality Control Processes	10(d)

CBI CROSS REFERENCE 3

Deleted Pages: Figure 13. DNA Sequence of the PCR Product from Conventional Control Ebony

Pages	Reason for Deletion	FIFRA Reference
52	Discloses Manufacturing or Quality Control Processes	10(d)

CBI CROSS REFERENCE 4

Deleted Pages: Figure 14. Alignment of the 5' Flanking Sequence of the MON 88302 Insert with the Conventional Control Ebony Sequence

Pages	Reason for Deletion	FIFRA Reference
53-54	Discloses Manufacturing or Quality Control Processes	10(d)

CBI CROSS REFERENCE 5

Deleted Pages: Figure 15. Alignment of the 3' Flanking Sequence of the MON 88302 Insert with the Conventional Control Ebony Sequence

Pages	Reason for Deletion	FIFRA Reference
55-56	Discloses Manufacturing or Quality Control Processes	10(d)

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   1 CATGCGTTTG CGGTATACTT AATATGCACG GGAATGATTC TGGCGGACGT TATCTATCTT
  61 TTTTTGTAGG TCCTAATAAA CGGGCTACAG CACTTTGTGC CAACAAAGGT GAAGCCAAAG
 121 TTACAGATAG TCGAAACATT TATCAAGGTA AGCAAACCAG AAACTCATAT GAAAGTATAG
 181 CAGACTTGAG ATCATAATAT GCTGGTGATA CACACTTAAA AATCGGAATC ATCACTCATT
 241 TTTTTTGCAG GCATACTATC TGCCAGAGAC GGAATATGTC CACTGGGCAA GAGCTCATCC
 301 GGTAAACAAA CAAATCTTTC TTAATCTTTC TTAATCTTTA TAATGTTTTG CGTAAATTAA
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 421 CACAGGTCAT TGGACTTGTG AATTTAGTAG CCACCATGAA AAGCTGGAAG AGGAAAACGC
 481 GTCTAGAAGT TGTGGATAAG ATTGAATCAG CTGCTGCATA GATCAAATCT AAAAGCAACA
 541 ACAAAGTAAT TTTTTTACTT CTTTTCTCTG GTCTTGTTCT GTCTTTGCTT TTGGCTCTTA
 601 CTTTTGCGTT TTGAACCGAG TGTGTAAATT TGAGGATAAG CCCTTCTTAG TTATCATCTT
 661 TCTTTTGCTT AATGGGGTTT GTGTAAAAGA TCCTCCTCAA GTTGTACAGT CTTGAAGAGA
 721 TTGTAACACA CGGTTTCCTA CATTTAAATA CTTAATTAAT GTCTCAGTAT TTGTATTATC
 781 AGTTCCTTGA ACCTTATTTT ATAGTGCACA AAACCTTTTA GTCATCATGT TGTACCACTT
 841 CAAACACTGA TAGTTTAAAC TGAAGGCGGG AAACGACAAT CTGATCCCCA TCAAGCTCTA
 901 GCTAGAGCGG CCGCGTTATC AAGCTTCTGC AGGTCCTGCT CGAGTGGAAG CTAATTCTCA
 961 GTCCAAAGCC TCAACAAGGT CAGGGTACAG AGTCTCCAAA CCATTAGCCA AAAGCTACAG
1021 GAGATCAATG AAGAATCTTC AATCAAAGTA AACTACTGTT CCAGCACATG CATCATGGTC
1081 AGTAAGTTTC AGAAAAAGAC ATCCACCGAA GACTTAAAGT TAGTGGGCAT CTTTGAAAGT
1141 AATCTTGTCA ACATCGAGCA GCTGGCTTGT GGGGACCAGA CAAAAAAGGA ATGGTGCAGA
1201 ATTGTTAGGC GCACCTACCA AAAGCATCTT TGCCTTTATT GCAAAGATAA AGCAGATTCC
1261 TCTAGTACAA GTGGGGAACA AAATAACGTG GAAAAGAGCT GTCCTGACAG CCCACTCACT
1321 AATGCGTATG ACGAACGCAG TGACGACCAC AAAAGAATTA GCTTGAGCTC AGGATTTAGC
1381 AGCATTCCAG ATTGGGTTCA ATCAACAAGG TACGAGCCAT ATCACTTTAT TCAAATTGGT
1441 ATCGCCAAAA CCAAGAAGGA ACTCCCATCC TCAAAGGTTT GTAAGGAAGA ATTCGATATC
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1561 GTTGTTATAA TAAACCATTT CCATTGTCAT GAGATTTTGA GGTTAATATA TACTTTACTT
1621 GTTCATTATT TTATTTGGTG TTTGAATAAA TGATATAAAT GGCTCTTGAT AATCTGCATT
1681 CATTGAGATA TCAAATATTT ACTCTAGAGA AGAGTGTCAT ATAGATTGAT GGTCCACAAT
1741 CAATGAAATT TTTGGGAGAC GAACATGTAT AACCATTTGC TTGAATAACC TTAATTAAAA
1801 GGTGTGATTA AATGATGTTT GTAACATGTA GTACTAAACA TTCATAAAAC ACAACCAACC
1861 CAAGAGGTAT TGAGTATTCA CGGCTAAACA GGGGCATAAT GGTAATTTAA AGAATGATAT
1921 TATTTTATGT TAAACCCTAA CATTGGTTTC GGATTCAACG CTATAAATAA AACCACTCTC
1981 GTTGCTGATT CCATTTATCG TTCTTATTGA CCCTAGCCGC TACACACTTT TCTGCGATAT
2041 CTCTGAGGTA AGCGTTAACG TACCCTTAGA TCGTTCTTTT TCTTTTTCGT CTGCTGATCG
2101 TTGCTCATAT TATTTCGATG ATTGTTGGAT TCGATGCTCT TTGTTGATTG ATCGTTCTGA
2161 AAATTCTGAT CTGTTGTTTA GATTTTATCG ATTGTTAATA TCAACGTTTC ACTGCTTCTA
2221 AACGATAATT TATTCATGAA ACTATTTTCC CATTCTGATC GATCTTGTTT TGAGATTTTA
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2821 TGAAGAAGAG TGGGATGACG TTAATTGGCT CTGAGCTTCG TCCTCTTAAG GTCATGTCTT
2881 CTGTTTCCAC GGCGTGCATG CTTCACGGTG CAAGCAGCCG TCCAGCAACT GCTCGTAAGT
2941 CCTCTGGTCT TTCTGGAACC GTCCGTATTC CAGGTGACAA GTCTATCTCC CACAGGTCCT
3001 TCATGTTTGG AGGTCTCGCT AGCGGTGAAA CTCGTATCAC CGGTCTTTTG GAAGGTGAAG
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3301 ACCCACTTCG CGAAATGGGT GTGCAGGTGA AGTCTGAAGA CGGTGATCGT CTTCCAGTTA
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3421 AAGTGAAGTC CGCTGTTCTG CTTGCTGGTC TCAACACCCC AGGTATCACC ACTGTTATCG
3481 AGCCAATCAT GACTCGTGAC CACACTGAAA AGATGCTTCA AGGTTTTGGT GCTAACCTTA
3541 CCGTTGAGAC TGATGCTGAC GGTGTGCGTA CCATCCGTCT TGAAGGTCGT GGTAAGCTCA
3601 CCGGTCAAGT GATTGATGTT CCAGGTGATC CATCCTCTAC TGCTTTCCCA TTGGTTGCTG
3661 CCTTGCTTGT TCCAGGTTCC GACGTCACCA TCCTTAACGT TTTGATGAAC CCAACCCGTA
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3721 CTGGTCTCAT CTTGACTCTG CAGGAAATGG GTGCCGACAT CGAAGTGATC AACCCACGTC
3781 TTGCTGGTGG AGAAGACGTG GCTGACTTGC GTGTTCGTTC TTCTACTTTG AAGGGTGTTA
3841 CTGTTCCAGA AGACCGTGCT CCTTCTATGA TCGACGAGTA TCCAATTCTC GCTGTTGCAG
3901 CTGCATTCGC TGAAGGTGCT ACCGTTATGA ACGGTTTGGA AGAACTCCGT GTTAAGGAAA
3961 GCGACCGTCT TTCTGCTGTC GCAAACGGTC TCAAGCTCAA CGGTGTTGAT TGCGATGAAG
4021 GTGAGACTTC TCTCGTCGTG CGTGGTCGTC CTGACGGTAA GGGTCTCGGT AACGCTTCTG
4081 GAGCAGCTGT CGCTACCCAC CTCGATCACC GTATCGCTAT GAGCTTCCTC GTTATGGGTC
4141 TCGTTTCTGA AAACCCTGTT ACTGTTGATG ATGCTACTAT GATCGCTACT AGCTTCCCAG
4201 AGTTCATGGA TTTGATGGCT GGTCTTGGAG CTAAGATCGA ACTCTCCGAC ACTAAGGCTG
4261 CTTGATGAGC TCAAGAATTC GAGCTCGGTA CCGGATCCTC TAGCTAGAGC TTTCGTTCGT
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4381 GAATCCTACT GAGTTTGAGT ATTATGGCAT TGGGAAAACT GTTTTTCTTG TACCATTTGT
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5281 CTTCAATTTT TTTTAATGTC ATTATGATAG ATGAATAATT CCTTTTCTTA TCCTGTTGAC
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6121 TAATAGAAAA ATAAAGAAAC TGCAGCAGGA AGATAAAGAA AACATGAGAG ACAT
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Figure 10. DNA Sequence of the Insert and Adjacent Genomic DNA in MON 88302

This DNA sequence represents the consensus sequence of overlapping PCR products spanning the insert in MON 88302 (Figure 9). Bases 1-839 (single-underlined) represent genomic DNA flanking the 5′ end of the insert. Bases 840-5267 represent the inserted DNA corresponding to bases 315-4742 from plasmid vector PV-BNHT2672 (Figure 11). Bases 5268-5276 (bolded) represent a 9 bp insertion immediately adjacent to the 3' end of the insert. Bases 5277-6174 (double-underlined) represent genomic DNA flanking the 3′ end of the insert. The specific base pair locations for the genetic elements within the MON 88302 insert and the 5' and 3' flanking sequences are described in Table 2.

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315   TCAAACACTGATAGTTTAAACTGAAGGCGGGAAACGACAATCTGATCCCCATCAAGCTCT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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      AGTCCAAAGCCTCAACAAGGTCAGGGTACAGAGTCTCCAAACCATTAGCCAAAAGCTACA
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AGTCCAAAGCCTCAACAAGGTCAGGGTACAGAGTCTCCAAACCATTAGCCAAAAGCTACA

      GGAGATCAATGAAGAATCTTCAATCAAAGTAAACTACTGTTCCAGCACATGCATCATGGT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      GGAGATCAATGAAGAATCTTCAATCAAAGTAAACTACTGTTCCAGCACATGCATCATGGT

      CAGTAAGTTTCAGAAAAAGACATCCACCGAAGACTTAAAGTTAGTGGGCATCTTTGAAAG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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      TAATCTTGTCAACATCGAGCAGCTGGCTTGTGGGGACCAGACAAAAAAGGAATGGTGCAG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TAATCTTGTCAACATCGAGCAGCTGGCTTGTGGGGACCAGACAAAAAAGGAATGGTGCAG

      AATTGTTAGGCGCACCTACCAAAAGCATCTTTGCCTTTATTGCAAAGATAAAGCAGATTC
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AATTGTTAGGCGCACCTACCAAAAGCATCTTTGCCTTTATTGCAAAGATAAAGCAGATTC

      CTCTAGTACAAGTGGGGAACAAAATAACGTGGAAAAGAGCTGTCCTGACAGCCCACTCAC
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      CTCTAGTACAAGTGGGGAACAAAATAACGTGGAAAAGAGCTGTCCTGACAGCCCACTCAC

      TAATGCGTATGACGAACGCAGTGACGACCACAAAAGAATTAGCTTGAGCTCAGGATTTAG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TAATGCGTATGACGAACGCAGTGACGACCACAAAAGAATTAGCTTGAGCTCAGGATTTAG

      CAGCATTCCAGATTGGGTTCAATCAACAAGGTACGAGCCATATCACTTTATTCAAATTGG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      CAGCATTCCAGATTGGGTTCAATCAACAAGGTACGAGCCATATCACTTTATTCAAATTGG

      TATCGCCAAAACCAAGAAGGAACTCCCATCCTCAAAGGTTTGTAAGGAAGAATTCGATAT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TATCGCCAAAACCAAGAAGGAACTCCCATCCTCAAAGGTTTGTAAGGAAGAATTCGATAT

      CAAGCTTGATATCGGAAGTTTCTCTCTTGAGGGAGGTTGCTCGTGGAATGGGACACATAT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      CAAGCTTGATATCGGAAGTTTCTCTCTTGAGGGAGGTTGCTCGTGGAATGGGACACATAT
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GGTTGTTATAATAAACCATTTCCATTGTCATGAGATTTTGAGGTTAATATATACTTTACT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGTTGTTATAATAAACCATTTCCATTGTCATGAGATTTTGAGGTTAATATATACTTTACT

TGTTCATTATTTTATTTGGTGTTTGAATAAATGATATAAATGGCTCTTGATAATCTGCAT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGTTCATTATTTTATTTGGTGTTTGAATAAATGATATAAATGGCTCTTGATAATCTGCAT

TCATTGAGATATCAAATATTTACTCTAGAGAAGAGTGTCATATAGATTGATGGTCCACAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCATTGAGATATCAAATATTTACTCTAGAGAAGAGTGTCATATAGATTGATGGTCCACAA

TCAATGAAATTTTTGGGAGACGAACATGTATAACCATTTGCTTGAATAACCTTAATTAAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCAATGAAATTTTTGGGAGACGAACATGTATAACCATTTGCTTGAATAACCTTAATTAAA

AGGTGTGATTAAATGATGTTTGTAACATGTAGTACTAAACATTCATAAAACACAACCAAC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGGTGTGATTAAATGATGTTTGTAACATGTAGTACTAAACATTCATAAAACACAACCAAC

CCAAGAGGTATTGAGTATTCACGGCTAAACAGGGGCATAATGGTAATTTAAAGAATGATA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCAAGAGGTATTGAGTATTCACGGCTAAACAGGGGCATAATGGTAATTTAAAGAATGATA

TTATTTTATGTTAAACCCTAACATTGGTTTCGGATTCAACGCTATAAATAAAACCACTCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTATTTTATGTTAAACCCTAACATTGGTTTCGGATTCAACGCTATAAATAAAACCACTCT

CGTTGCTGATTCCATTTATCGTTCTTATTGACCCTAGCCGCTACACACTTTTCTGCGATA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CGTTGCTGATTCCATTTATCGTTCTTATTGACCCTAGCCGCTACACACTTTTCTGCGATA

TCTCTGAGGTAAGCGTTAACGTACCCTTAGATCGTTCTTTTTCTTTTTCGTCTGCTGATC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCTCTGAGGTAAGCGTTAACGTACCCTTAGATCGTTCTTTTTCTTTTTCGTCTGCTGATC

GTTGCTCATATTATTTCGATGATTGTTGGATTCGATGCTCTTTGTTGATTGATCGTTCTG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTTGCTCATATTATTTCGATGATTGTTGGATTCGATGCTCTTTGTTGATTGATCGTTCTG

AAAATTCTGATCTGTTGTTTAGATTTTATCGATTGTTAATATCAACGTTTCACTGCTTCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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AAACGATAATTTATTCATGAAACTATTTTCCCATTCTGATCGATCTTGTTTTGAGATTTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAACGATAATTTATTCATGAAACTATTTTCCCATTCTGATCGATCTTGTTTTGAGATTTT

AATTTGTTCGATTGATTGTTGGTTGGTGGATCTATATACGAGTGAACTTGTTGATTTGCG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AATTTGTTCGATTGATTGTTGGTTGGTGGATCTATATACGAGTGAACTTGTTGATTTGCG
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TATTTAAGATGTATGTCGATTTGAATTGTGATTGGGTAATTCTGGAGTAGCATAACAAAT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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CCAGTGTTCCCTTTTTCTAAGGGTAATTCTCGGATTGTTTGCTTTATATCTCTTGAAATT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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GCCGATTTGATTGAATTTAGCTCGCTTAGCTCAGATGATAGAGCACCACAATTTTTGTGG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCCGATTTGATTGAATTTAGCTCGCTTAGCTCAGATGATAGAGCACCACAATTTTTGTGG

TAGAAATCGGTTTGACTCCGATAGCGGCTTTTTACTATGATTGTTTTGTGTTAAAGATGA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TAGAAATCGGTTTGACTCCGATAGCGGCTTTTTACTATGATTGTTTTGTGTTAAAGATGA

TTTTCATAATGGTTATATATGTCTACTGTTTTTATTGATTCAATATTTGATTGTTCTTTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTTCATAATGGTTATATATGTCTACTGTTTTTATTGATTCAATATTTGATTGTTCTTTT

TTTTGCAGATTTGTTGACCAGAGATCTACCATGGCGCAAGTTAGCAGAATCTGCAATGGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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GTGCAGAACCCATCTCTTATCTCCAATCTCTCGAAATCCAGTCAACGCAAATCTCCCTTA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTGCAGAACCCATCTCTTATCTCCAATCTCTCGAAATCCAGTCAACGCAAATCTCCCTTA

TCGGTTTCTCTGAAGACGCAGCAGCATCCACGAGCTTATCCGATTTCGTCGTCGTGGGGA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCGGTTTCTCTGAAGACGCAGCAGCATCCACGAGCTTATCCGATTTCGTCGTCGTGGGGA

TTGAAGAAGAGTGGGATGACGTTAATTGGCTCTGAGCTTCGTCCTCTTAAGGTCATGTCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTGAAGAAGAGTGGGATGACGTTAATTGGCTCTGAGCTTCGTCCTCTTAAGGTCATGTCT

TCTGTTTCCACGGCGTGCATGCTTCACGGTGCAAGCAGCCGTCCAGCAACTGCTCGTAAG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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TCCTCTGGTCTTTCTGGAACCGTCCGTATTCCAGGTGACAAGTCTATCTCCCACAGGTCC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TCCTCTGGTCTTTCTGGAACCGTCCGTATTCCAGGTGACAAGTCTATCTCCCACAGGTCC

TTCATGTTTGGAGGTCTCGCTAGCGGTGAAACTCGTATCACCGGTCTTTTGGAAGGTGAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTCATGTTTGGAGGTCTCGCTAGCGGTGAAACTCGTATCACCGGTCTTTTGGAAGGTGAA

GATGTTATCAACACTGGTAAGGCTATGCAAGCTATGGGTGCCAGAATCCGTAAGGAAGGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GATGTTATCAACACTGGTAAGGCTATGCAAGCTATGGGTGCCAGAATCCGTAAGGAAGGT
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GATACTTGGATCATTGATGGTGTTGGTAACGGTGGACTCCTTGCTCCTGAGGCTCCTCTC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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GATTTCGGTAACGCTGCAACTGGTTGCCGTTTGACTATGGGTCTTGTTGGTGTTTACGAT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GATTTCGGTAACGCTGCAACTGGTTGCCGTTTGACTATGGGTCTTGTTGGTGTTTACGAT

TTCGATAGCACTTTCATTGGTGACGCTTCTCTCACTAAGCGTCCAATGGGTCGTGTGTTG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTCGATAGCACTTTCATTGGTGACGCTTCTCTCACTAAGCGTCCAATGGGTCGTGTGTTG

AACCCACTTCGCGAAATGGGTGTGCAGGTGAAGTCTGAAGACGGTGATCGTCTTCCAGTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AACCCACTTCGCGAAATGGGTGTGCAGGTGAAGTCTGAAGACGGTGATCGTCTTCCAGTT

ACCTTGCGTGGACCAAAGACTCCAACGCCAATCACCTACAGGGTACCTATGGCTTCCGCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCTTGCGTGGACCAAAGACTCCAACGCCAATCACCTACAGGGTACCTATGGCTTCCGCT

CAAGTGAAGTCCGCTGTTCTGCTTGCTGGTCTCAACACCCCAGGTATCACCACTGTTATC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CAAGTGAAGTCCGCTGTTCTGCTTGCTGGTCTCAACACCCCAGGTATCACCACTGTTATC

GAGCCAATCATGACTCGTGACCACACTGAAAAGATGCTTCAAGGTTTTGGTGCTAACCTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAGCCAATCATGACTCGTGACCACACTGAAAAGATGCTTCAAGGTTTTGGTGCTAACCTT

ACCGTTGAGACTGATGCTGACGGTGTGCGTACCATCCGTCTTGAAGGTCGTGGTAAGCTC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCGTTGAGACTGATGCTGACGGTGTGCGTACCATCCGTCTTGAAGGTCGTGGTAAGCTC

ACCGGTCAAGTGATTGATGTTCCAGGTGATCCATCCTCTACTGCTTTCCCATTGGTTGCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACCGGTCAAGTGATTGATGTTCCAGGTGATCCATCCTCTACTGCTTTCCCATTGGTTGCT

GCCTTGCTTGTTCCAGGTTCCGACGTCACCATCCTTAACGTTTTGATGAACCCAACCCGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCCTTGCTTGTTCCAGGTTCCGACGTCACCATCCTTAACGTTTTGATGAACCCAACCCGT

ACTGGTCTCATCTTGACTCTGCAGGAAATGGGTGCCGACATCGAAGTGATCAACCCACGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACTGGTCTCATCTTGACTCTGCAGGAAATGGGTGCCGACATCGAAGTGATCAACCCACGT

CTTGCTGGTGGAGAAGACGTGGCTGACTTGCGTGTTCGTTCTTCTACTTTGAAGGGTGTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CTTGCTGGTGGAGAAGACGTGGCTGACTTGCGTGTTCGTTCTTCTACTTTGAAGGGTGTT

ACTGTTCCAGAAGACCGTGCTCCTTCTATGATCGACGAGTATCCAATTCTCGCTGTTGCA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACTGTTCCAGAAGACCGTGCTCCTTCTATGATCGACGAGTATCCAATTCTCGCTGTTGCA
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GCTGCATTCGCTGAAGGTGCTACCGTTATGAACGGTTTGGAAGAACTCCGTGTTAAGGAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCTGCATTCGCTGAAGGTGCTACCGTTATGAACGGTTTGGAAGAACTCCGTGTTAAGGAA

AGCGACCGTCTTTCTGCTGTCGCAAACGGTCTCAAGCTCAACGGTGTTGATTGCGATGAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGCGACCGTCTTTCTGCTGTCGCAAACGGTCTCAAGCTCAACGGTGTTGATTGCGATGAA

GGTGAGACTTCTCTCGTCGTGCGTGGTCGTCCTGACGGTAAGGGTCTCGGTAACGCTTCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGTGAGACTTCTCTCGTCGTGCGTGGTCGTCCTGACGGTAAGGGTCTCGGTAACGCTTCT

GGAGCAGCTGTCGCTACCCACCTCGATCACCGTATCGCTATGAGCTTCCTCGTTATGGGT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GGAGCAGCTGTCGCTACCCACCTCGATCACCGTATCGCTATGAGCTTCCTCGTTATGGGT

CTCGTTTCTGAAAACCCTGTTACTGTTGATGATGCTACTATGATCGCTACTAGCTTCCCA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CTCGTTTCTGAAAACCCTGTTACTGTTGATGATGCTACTATGATCGCTACTAGCTTCCCA

GAGTTCATGGATTTGATGGCTGGTCTTGGAGCTAAGATCGAACTCTCCGACACTAAGGCT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GAGTTCATGGATTTGATGGCTGGTCTTGGAGCTAAGATCGAACTCTCCGACACTAAGGCT

GCTTGATGAGCTCAAGAATTCGAGCTCGGTACCGGATCCTCTAGCTAGAGCTTTCGTTCG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GCTTGATGAGCTCAAGAATTCGAGCTCGGTACCGGATCCTCTAGCTAGAGCTTTCGTTCG

TATCATCGGTTTCGACAACGTTCGTCAAGTTCAATGCATCAGTTTCATTGCGCACACACC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TATCATCGGTTTCGACAACGTTCGTCAAGTTCAATGCATCAGTTTCATTGCGCACACACC

AGAATCCTACTGAGTTTGAGTATTATGGCATTGGGAAAACTGTTTTTCTTGTACCATTTG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AGAATCCTACTGAGTTTGAGTATTATGGCATTGGGAAAACTGTTTTTCTTGTACCATTTG

TTGTGCTTGTAATTTACTGTGTTTTTTATTCGGTTTTCGCTATCGAACTGTGAAATGGAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTGTGCTTGTAATTTACTGTGTTTTTTATTCGGTTTTCGCTATCGAACTGTGAAATGGAA

ATGGATGGAGAAGAGTTAATGAATGATATGGTCCTTTTGTTCATTCTCAAATTAATATTA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATGGATGGAGAAGAGTTAATGAATGATATGGTCCTTTTGTTCATTCTCAAATTAATATTA

TTTGTTTTTTCTCTTATTTGTTGTGTGTTGAATTTGAAATTATAAGAGATATGCAAACAT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTGTTTTTTCTCTTATTTGTTGTGTGTTGAATTTGAAATTATAAGAGATATGCAAACAT

TTTGTTTTGAGTAAAAATGTGTCAAATCGTGGCCTCTAATGACCGAAGTTAATATGAGGA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTGTTTTGAGTAAAAATGTGTCAAATCGTGGCCTCTAATGACCGAAGTTAATATGAGGA
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GTAAAACACTTGTAGTTGTACCATTATGCTTATTCACTAGGCAACAAATATATTTTCAGA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
GTAAAACACTTGTAGTTGTACCATTATGCTTATTCACTAGGCAACAAATATATTTTCAGA

CCTAGAAAAGCTGCAAATGTTACTGAATACAAGTATGTCCTCTTGTGTTTTAGACATTTA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CCTAGAAAAGCTGCAAATGTTACTGAATACAAGTATGTCCTCTTGTGTTTTAGACATTTA

TGAACTTTCCTTTATGTAATTTTCCAGAATCCTTGTCAGATTCTAATCATTGCTTTATAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TGAACTTTCCTTTATGTAATTTTCCAGAATCCTTGTCAGATTCTAATCATTGCTTTATAA

TTATAGTTATACTCATGGATTTGTAGTTGAGTATGAAAATATTTTTTAATGCATTTTATG
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTATAGTTATACTCATGGATTTGTAGTTGAGTATGAAAATATTTTTTAATGCATTTTATG

ACTTGCCAATTGATTGACAACATGCATCAATCGACCTGCAGCCACTCGAAGCGGCCGCAT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ACTTGCCAATTGATTGACAACATGCATCAATCGACCTGCAGCCACTCGAAGCGGCCGCAT

CGATCGTGAAGTTTCTCATCTAAGCCCCCATTTGGACGTGAATGTAGACACGTCGAAATA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
CGATCGTGAAGTTTCTCATCTAAGCCCCCATTTGGACGTGAATGTAGACACGTCGAAATA

AAGATTTCCGAATTAGAATAATTTGTTTATTGCTTTCGCCTATAAATACGACGGATCGTA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAGATTTCCGAATTAGAATAATTTGTTTATTGCTTTCGCCTATAAATACGACGGATCGTA

ATTTGTCGTTTTATCAAAATGTACTTTCATTTTATAATAACGCTGCGGACATCTACATTT
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
ATTTGTCGTTTTATCAAAATGTACTTTCATTTTATAATAACGCTGCGGACATCTACATTT

TTGAATTGAAAAAAAATTGGTAATTACTCTTTCTTTTTCTCCATATTGACCATCATACTC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTGAATTGAAAAAAAATTGGTAATTACTCTTTCTTTTTCTCCATATTGACCATCATACTC

ATTGCTGATCCATGTAGATTTCCCGGACATGAAGCCATTTACAATTGA 4742
|||||||||||||||||||||||||||||||||||||||||||||||
ATTGCTGATCCATGTAGATTTCCCGGACATGAAGCCATTTACAATTGA 5267
```

Figure 11. Alignment of the MON 88302 Sequence with the Sequence of Plasmid Vector PV-BNHT2672

The T-DNA insert in MON 88302 (Figure 10, base 840-5267) represented by the bottom sequence in this figure, is identical to the corresponding T-DNA sequence from the plasmid vector PV-BNHT2672 (base 315-4742), which is represented by the top sequence.

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   1 TATACTTAAT ATGCACGGGA ATGATTCTGG CGGACGTTAT CTATCTTTTT TTGTAGGTCC
  61 TAATAAACGG GCTACAGCAC TTTGTGCCAA CAAAGGTGAA GCCAAAGTTA CAGATAGTCG
 121 AAACATTTAT CAAGGTAAGC AAACCAGAAA CTCATATGAA AGTATAGCAG ACTTGAGATC
 181 ATAATATGCT GGTGATACAC ACTTAAAAAT CGGAATCATC ACTCATTTTT TTTGCAGGCA
 241 TACTATCTGC CAGAGACGGA ATATGTCCAC TGGGCAAGAG CTCATCCGGT AAACAAACAA
 301 ATCTTTCTTA ATCTTTCTTA ATCTTTATAA TGTTTTGCGT AAATTAAATC GATGGGATAG
 361 AAGACTAATA TGATTAAAAT GTGTAAACAT ACAGGAATAT ACGAAAGCAC AGGTCATTGG
 421 ACTTGTGAAT TTAGTAGCCA CCATGAAAAG CTGGAAGAGG AAAACGCGTC TAGAAGTTGT
 481 GGATAAGATT GAATCAGCTG CTGCATAGAT CAAATCTAAA AGCAACAACA AAGTAATTTT
 541 TTTACTTCTT TTCTCTGGTC TTGTTCTGTC TTTGCTTTTG GCTCTTACTT TTGCGTTTTG
 601 AACCGAGTGT GTAAATTTGA GGATAAGCCC TTCTTAGTTA TCATCTTTCT TTTGCTTAAT
 661 GGGGTTTGTG TAAAAGATCC TCCTCAAGTT GTACAGTCTT GAAGAGATTG TAACACACGG
 721 TTTCCTACAT TTAAATACTT AATTAATGTC TCAGTATTTG TATTATCAGT TCCTTGAACC
 781 TTATTTTATA GTGCACAAAA CCTTTTAGTC ATCATGTTGT ACCACTTTAT GCTTTTGGAT
 841 ACTAATTAAC ACTCATCAAC TTCAATTTTT TTTAATGTCA TTATGATAGA TGAATAATTC
 901 CTTTTCTTAT CCTGTTGACC ATAATAATGA TAAAACAGTA ATCATGATAA ATATATCAAA
 961 AAGTGTATTT TAAAAATTTT CTAATCATTA TTCGAGAAAA AAAACAAATC ATTTGTAAGT
1021 TTCACTGTTA ACTACAGATG AAACATCTTT TGTTTTTAAC GTTTTAATGA TATTGAAATC
1081 AATCAGAATA AAAGGTGTCT CATCTCTTGT GTACTGTCAT GTTTGCGATG AGAGGCTGGA
1141 AGAAAGACTA GTCAAAAGAC TTCAAAGCTG TGGTGATTTA GTTGTATCTC CAACCATTTT
1201 TAATGCAACG CATGGTTCAT CTACTGGTAC CTGTTGCACA ATAAAACATT CAAAAACATG
1261 TTATTTTACA AATCTTCACT AAAAGCCTTC AATTTCATAT GTTGCGTGTC AATGTAAACC
1321 GACTTCTTAT TTTCAAACTG TTGATGTGAA AGAGAGAAAA AAATCAGAGA AGTAAAATTT
1381 ATGAAGGAGA TTTACTAAGA AGTAAATTGT TTTATAAATA ATTATTTTTA TATAAAATAA
1441 GATTTATTAT TATTTTTTCA TGTTAATGTT AAAAGACCTT TAAAAAATAT GCATTACGTT
1501 TTTATAACAG TAGAGATGTT TTTAAGAATT GATATTAGGT AGCTTTTTAA AATTAATTTA
1561 GTAATTATCT AAAAAAAATG ATCTCTTTTA TAAAAAGAAA AAATCACAGA GATCCAGATA
1621 CTGTCGTGTG AAGTATTAAA GAGATGTCTT TAAAAAGTAT TAAAGAGACA GTCGACAGTT
1681 TGCTATCTGT TGTAATAAAT AATAGAAAAA TAAAGAAACT GCAGCAGGAA GATAAAGAAA
1741 ACATGAGAGA CATAACACAA AAGAGACTTG TGCTTTCGTT TAGTAGATGC ATCACGTATA
1801 TG
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Figure 13. DNA Sequence of the PCR Product from Conventional Control Ebony

Bases 1-826 (single-underlined) represent conventional control genomic DNA sequence that is identical to the genomic DNA sequence flanking the 5' end of the MON 88302 insert (Figure 14). Bases 827-855 (bold) represent 29 bp of the conventional control genomic DNA not present in MON 88302. Bases 856-1753 (double-underlined) represent conventional control genomic DNA sequence that is identical to the genomic DNA sequence flanking the 3' end of the MON 88302 insert, except for one base pair difference (Figure 15). The DNA sequences of the oligonucleotide primers, Primer A and Primer B (Figure 12), which were used to generate the PCR product, are not included in the DNA sequence presented above since the primers used to amplify the product were also used to sequence the product. Therefore, the sequence data adjacent to the primer sites was not obtained. The primer sequences were as follows:

Primer A: 5' CAC TGT TAT TTT CCT TTC CAC TAG ATG C 3'
Primer B: 5' CAT CCG TAT TTA TTG AGT ATT GTT GAA TAA 3'

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14      TATACTTAATATGCACGGGAATGATTCTGGCGGACGTTATCTATCTTTTTTTGTAGGTCC
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
1       TATACTTAATATGCACGGGAATGATTCTGGCGGACGTTATCTATCTTTTTTTGTAGGTCC

        TAATAAACGGGCTACAGCACTTTGTGCCAACAAAGGTGAAGCCAAAGTTACAGATAGTCG
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        TAATAAACGGGCTACAGCACTTTGTGCCAACAAAGGTGAAGCCAAAGTTACAGATAGTCG

        AAACATTTATCAAGGTAAGCAAACCAGAAACTCATATGAAAGTATAGCAGACTTGAGATC
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        AAACATTTATCAAGGTAAGCAAACCAGAAACTCATATGAAAGTATAGCAGACTTGAGATC

        ATAATATGCTGGTGATACACACTTAAAAATCGGAATCATCACTCATTTTTTTTGCAGGCA
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        ATAATATGCTGGTGATACACACTTAAAAATCGGAATCATCACTCATTTTTTTTGCAGGCA

        TACTATCTGCCAGAGACGGAATATGTCCACTGGGCAAGAGCTCATCCGGTAAACAAACAA
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        TACTATCTGCCAGAGACGGAATATGTCCACTGGGCAAGAGCTCATCCGGTAAACAAACAA

        ATCTTTCTTAATCTTTCTTAATCTTTATAATGTTTTGCGTAAATTAAATCGATGGGATAG
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        ATCTTTCTTAATCTTTCTTAATCTTTATAATGTTTTGCGTAAATTAAATCGATGGGATAG

        AAGACTAATATGATTAAAATGTGTAAACATACAGGAATATACGAAAGCACAGGTCATTGG
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        AAGACTAATATGATTAAAATGTGTAAACATACAGGAATATACGAAAGCACAGGTCATTGG

        ACTTGTGAATTTAGTAGCCACCATGAAAAGCTGGAAGAGGAAAACGCGTCTAGAAGTTGT
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        ACTTGTGAATTTAGTAGCCACCATGAAAAGCTGGAAGAGGAAAACGCGTCTAGAAGTTGT

        GGATAAGATTGAATCAGCTGCTGCATAGATCAAATCTAAAAGCAACAACAAAGTAATTTT
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        GGATAAGATTGAATCAGCTGCTGCATAGATCAAATCTAAAAGCAACAACAAAGTAATTTT

        TTTACTTCTTTTCTCTGGTCTTGTTCTGTCTTTGCTTTTGGCTCTTACTTTTGCGTTTTG
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        TTTACTTCTTTTCTCTGGTCTTGTTCTGTCTTTGCTTTTGGCTCTTACTTTTGCGTTTTG

        AACCGAGTGTGTAAATTTGAGGATAAGCCCTTCTTAGTTATCATCTTTCTTTTGCTTAAT
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        AACCGAGTGTGTAAATTTGAGGATAAGCCCTTCTTAGTTATCATCTTTCTTTTGCTTAAT

        GGGGTTTGTGTAAAAGATCCTCCTCAAGTTGTACAGTCTTGAAGAGATTGTAACACACGG
        ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
        GGGGTTTGTGTAAAAGATCCTCCTCAAGTTGTACAGTCTTGAAGAGATTGTAACACACGG
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TTTCCTACATTTAAATACTTAATTAATGTCTCAGTATTTGTATTATCAGTTCCTTGAACC
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTTCCTACATTTAAATACTTAATTAATGTCTCAGTATTTGTATTATCAGTTCCTTGAACC

TTATTTTATAGTGCACAAAACCTTTTAGTCATCATGTTGTACCACT 839
|||||||||||||||||||||||||||||||||||||||||||||
TTATTTTATAGTGCACAAAACCTTTTAGTCATCATGTTGTACCACT 826
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Figure 14. Alignment of the 5' Flanking Sequence of the MON 88302 Insert with the Conventional Control Ebony Sequence

The DNA sequence immediately flanks the 5' end of the MON 88302 insert (Figure 10, bases14-839) represented by the top sequence is identical to base 1 to base 826 of the conventional control sequence (Figure 13), which is represented by the bottom sequence.

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5277  TCAACTTCAATTTTTTTTAATGTCATTATGATAGATGAATAATTCCTTTTCTTATCCTGT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
856   TCAACTTCAATTTTTTTTAATGTCATTATGATAGATGAATAATTCCTTTTCTTATCCTGT

      TGACCATAATAATGATAAAACAGTAATCATGATAAATATATCAAAAAGTGTATTTTAAAA
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TGACCATAATAATGATAAAACAGTAATCATGATAAATATATCAAAAAGTGTATTTTAAAA

      ATTTTCTAATCATTATTCGAGAAAAAAAAACAAATCATTTGTAAGTTTCACTGTTAACTAC
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      ATTTTCTAATCATTATTCGAGAAAAAAAAACAAATCATTTGTAAGTTTCACTGTTAACTAC

      AGATGAAACATCTTTTGTTTTTAACGTTTTAATGATATTGAAATCAATCAGAATAAAAGG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AGATGAAACATCTTTTGTTTTTAACGTTTTAATGATATTGAAATCAATCAGAATAAAAGG

      TGTCTCATCTCTTGTGTACTGTCATGTTTGCGATGAGAGGCTGGAAGAAAGACTAGTCAA
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TGTCTCATCTCTTGTGTACTGTCATGTTTGCGATGAGAGGCTGGAAGAAAGACTAGTCAA

      AAGACTTCAAAGCTGTGGTGATTTAGTTGTATCTCCAACCATTTTTAATGCAACGCATGG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AAGACTTCAAAGCTGTGGTGATTTAGTTGTATCTCCAACCATTTTTAATGCAACGCATGG

      TTCATCTACTGGTACCTGTTGCACAATAAAACATTCAAAAACATGTTATTTTACAAATCT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TTCATCTACTGGTACCTGTTGCACAATAAAACATTCAAAAACATGTTATTTTACAAATCT

      TCACTAAAAGCCTTCAATTTCATATGATGCGTGTCAATGTAAACCGACTTCTTATTTTCA
      |||||||||||||||||||||||||  ||||||||||||||||||||||||||||||||
      TCACTAAAAGCCTTCAATTTCATATGTTGCGTGTCAATGTAAACCGACTTCTTATTTTCA

      AACTGTTGATGTGAAAGAGAGAAAAAAATCAGAGAAGTAAAATTTATGAAGGAGATTTAC
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AACTGTTGATGTGAAAGAGAGAAAAAAATCAGAGAAGTAAAATTTATGAAGGAGATTTAC

      TAAGAAGTAAATTGTTTTATAAATAATTATTTTTATATAAAATAAGATTTATTATTATTT
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TAAGAAGTAAATTGTTTTATAAATAATTATTTTTATATAAAATAAGATTTATTATTATTT

      TTTCATGTTAATGTTAAAAGACCTTTAAAAAATATGCATTACGTTTTTATAACAGTAGAG
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TTTCATGTTAATGTTAAAAGACCTTTAAAAAATATGCATTACGTTTTTATAACAGTAGAG

      ATGTTTTTAAGAATTGATATTAGGTAGCTTTTTAAAATTAATTTAGTAATTATCTAAAAA
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      ATGTTTTTAAGAATTGATATTAGGTAGCTTTTTAAAATTAATTTAGTAATTATCTAAAAA
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AAATGATCTCTTTTATAAAAAGAAAAAATCACAGAGATCCAGATACTGTCGTGTGAAGTA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
AAATGATCTCTTTTATAAAAAGAAAAAATCACAGAGATCCAGATACTGTCGTGTGAAGTA

TTAAAGAGATGTCTTTAAAAAGTATTAAAGAGACAGTCGACAGTTTGCTATCTGTTGTAA
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TTAAAGAGATGTCTTTAAAAAGTATTAAAGAGACAGTCGACAGTTTGCTATCTGTTGTAA

TAAATAATAGAAAAATAAAGAAACTGCAGCAGGAAGATAAAGAAAACATGAGAGACAT 6174
||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
TAAATAATAGAAAAATAAAGAAACTGCAGCAGGAAGATAAAGAAAACATGAGAGACAT 1753
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Figure 15. Alignment of the 3' Flanking Sequence of the MON 88302 Insert with the Conventional Control Ebony Sequence

The DNA sequence flanking the 3' end of the MON 88302 insert (Figure 10, bases 5277-6174) is represented by the top sequence. The bottom sequence consists of base 856 to base 1753 of the conventional control sequence (Figure 13). Those two sequences are identical except for one base pair difference between the MON 88302 sequence at position 5723 and the conventional control sequence at position 1302.