

Notices

Federal Register

Vol. 61, No. 52

Friday, March 15, 1996

This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 96-006-1]

Monsanto Co.; Addition of Two Genetically Engineered Insect Resistant Corn Lines to Determination of Nonregulated Status

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: The Animal and Plant Health Inspection Service is announcing that it has added two additional genetically engineered, insect resistant corn lines to its August 22, 1995, determination that the Monsanto Company's corn line MON 80100 need no longer be regulated. The effect of this action is that two additional insect resistant corn lines designated as MON 809 and MON 810, which have been modified by the incorporation of genetic material described by the Monsanto Company, will no longer be subject to regulation under 7 CFR part 340.

FOR FURTHER INFORMATION CONTACT: Dr. Ved Malik, Biotechnologist, Animal and Plant Health Inspection Service, Biotechnology, Biologics, and Environmental Protection, Biotechnology Permits, 4700 River Road Unit 147, Riverdale, MD 20737-1237; (301) 734-7612.

SUPPLEMENTARY INFORMATION: On September 5, 1995, the Animal and Plant Health Inspection Service (APHIS) published a notice in the Federal Register (60 FR 46107-46108, Docket No. 95-041-2) announcing the issuance of a determination effective August 22, 1995, that an insect resistant corn line developed by the Monsanto Company (Monsanto) designated as corn line MON 80100, does not present a plant pest risk and is not a regulated article under the regulations contained in 7

CFR part 340. This action was in response to a petition submitted by Monsanto seeking a determination from APHIS that its corn line MON 80100 no longer be deemed a regulated article, based on an absence of plant pest risk. The effect of that action was that the subject corn line and its progeny would no longer be regulated under the regulations in 7 CFR part 340.

The two additional corn lines that are the subject of this notice, MON 809 and MON 810, were identified in Monsanto's previously submitted petition (APHIS Petition No. 95-093-01p) for corn line MON 80100. On January 17, 1996, APHIS received additional information and field test data in a petition (APHIS Petition No. 96-017-01p) in support of nonregulated status under 7 CFR part 340 for corn lines MON 809 and MON 810. As described by Monsanto, corn lines MON 809 and MON 810 express a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* which confers resistance to European corn borer. The subject corn lines were generated through use of the particle acceleration transformation system to insert plasmid vectors PV-ZMBK07 and PV-ZMGT10, the same vectors used to transform corn line MON 80100 for which the August 22, 1995, determination of nonregulated status was issued by APHIS.

Corn lines MON 809 and MON 810 have been evaluated in field tests conducted in 1993 and 1994 under APHIS permits and notifications. Reports from field trials and other data indicate that the subject corn lines grow normally, exhibit the expected morphological, reproductive, and physiological properties, and do not have unexpected pest or disease susceptibility or symptoms. Therefore, the APHIS determination of nonregulated status of August 22, 1995, applies as well to Monsanto's two new transformed corn lines, MON 809 and MON 810.

Done in Washington, DC, this 11th day of March 1996.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 96-6201 Filed 3-14-96; 8:45 am]

BILLING CODE 3410-34-P

96-017-01p
additional lines
see 95-093-01p

Monsanto

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198
Phone: (314) 694-1000

February 13, 1995

Dr. Vedpal Malik
Biotechnology Permit Unit
Biotechnology, Biologics and Environmental Protection
USDA-APHIS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

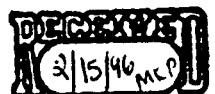
Dear Dr. Malik:

Please find enclosed the Southern data in support of the molecular analysis for YieldGard corn lines MON 809 and 810. This additional information is submitted in support of Monsanto's USDA petition for non-regulated status for additional corn lines MON 809 and MON 810 received by the USDA on January 17, 1996 and identified as petition 96-017-01p. These lines were previously identified in USDA petition 95-093-01p which provided non-regulated status for line MON 80100 dated August 22, 1995 (FR 60:171; pp. 46107-46108). Approval of lines MON 809 and 810 has been requested in connection with this previous approval.

Sincerely,



Kent A. Croon, Ph.D.
Regulatory Affairs Manager



Molecular Analysis of YieldGard™ Corn Line MON 809

Janice Kania, Pamela Keck and Patricia Sanders

I. SUMMARY

This report describes the molecular analysis of the integrated DNA (I-DNA) present in YieldGard™ corn line MON 809. Specifically, the insert number (number of integration sites within the corn genome) and the number and integrity of each inserted gene were determined. The corn line MON 809 was produced by particle acceleration technology with two plasmids PV-ZMBK07 [*cryIA(b)* gene] and PV-ZMGT10 [CP4 EPSPS and *gox* genes]. Corn line MON 809 contains one I-DNA of approximately 23 Kb which includes either complete or partial genes of *cryIA(b)*, CP4 EPSPS and *gox*. The I-DNA contains two *cryIA(b)* genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). There are two CP4 EPSPS genes, both of expected size (1.3 Kb). The single *gox* gene present in corn line MON 809 is not intact. The *nptII* and *ori-pUC* probings showed that the backbone was present in the YieldGard™ corn line MON 809, but was not the predicted size. Based on these analyses, we conclude that corn line MON 809 contains a single I-DNA with an intact *cryIA(b)* gene and two CP4 EPSPS genes that are responsible for producing the correct size CryIA(b) and CP4 EPSPS proteins.

Summary of Corn Line MON 809 Molecular Analysis

<u>Genetic Element</u>	<u>23 Kb insert</u>
<i>cryIA(b)</i> gene	1 full length, 1 partial
CP4 EPSPS gene	2 full length
<i>gox</i> gene	1 partial
<i>nptII/ori-pUC</i>	present

II. RESULTS AND DISCUSSION

A. Southern blot results

Two plasmid vectors were utilized during the particle acceleration process to produce the corn line MON 809. Plasmid PV-ZMBK07 contained the *cryIA(b)* gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and *gox* genes. The maps of the two plasmid vectors are presented in Figure 1, along with the locations of the restriction sites utilized for Southern analyses.

The DNAs from MON 818 and MON 809 plants were digested with a variety of restriction enzymes and subjected to Southern blot hybridization analyses to characterize the DNA that was stably transferred during the particle acceleration into the corn genome. Specifically, the insert number (number of integration sites within the corn genome), and the copy number and integrity of each inserted gene was examined.

B. Insert Number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the corn line MON 809. The plasmids PV-ZMBK07 and PV-ZMGT10 do not contain a restriction site for NdeI. Thus this enzyme effectively cuts outside any inserted DNA, releasing a fragment containing the inserted DNA. MON 818 control DNA and MON 809 DNA were digested with NdeI and probed with the *cryIA(b)* gene, the CP4 EPSPS gene and the *gox* gene. The results are shown in Figure 2. Lanes 1, 3 and 5 contain MON 818 control DNA. No bands were observed, as expected, when probed with the *cryIA(b)*, CP4 EPSPS or *gox* genes. MON 809 DNA produced one band, approximately 23 Kb in size, when probed with: the *cryIA(b)* gene (lane 2), the CP4 EPSPS gene (lane 4) and the *gox* gene (lane 6). The band produced in the *gox* gene probing is very faint and only observed with long exposure times, suggesting that only a portion of the *gox* gene is present in the inserted DNA of YieldGard™ corn line MON 809.

C. Insert Composition

1. *cryIA(b)* gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the *cryIA(b)* gene in MON 809 and the Southern blot probed with the *cryIA(b)* gene. The results are shown in Figure 3, lanes 1-3. The MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 (lane 2). The MON 818 DNA (lane 1) produces two faint bands, approximately 2.5 Kb and 1.9 Kb in size. These bands are considered to be background bands since they are observed in all three lanes and are not discussed further. The MON 818 DNA mixed with plasmids (lane 2) produced one new 3.46 Kb fragment which corresponds to the expected size of the intact *cryIA(b)* gene (refer to the PV-ZMBK07 plasmid map in Fig. 1). The MON 809 DNA (lane 3) contains two bands, 3.46 Kb and 1.0 Kb. The 3.46 Kb band is the expected size band for an intact *cryIA(b)* gene, and the 1.0 Kb band represents a partial *cryIA(b)* gene. The NcoI/EcoRI digests, probed with the *cryIA(b)* gene, identified one intact and one partial *cryIA(b)* gene.

2. CP4 EPSPS gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the CP4 EPSPS gene in MON 809 DNA and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure 4, lanes 1-3. The MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 also digested with NcoI/EcoRI (lane 2). The MON 818 DNA (lane 1) showed two bands, approximately 1.37 Kb and 0.80 Kb in size. These two bands, present in all three lanes, are background bands and are therefore not considered further. The MON 818 DNA mixed with the plasmids (lane 2) produced an additional band, 1.06 Kb, which is the expected size of the CP4 EPSPS gene, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). The MON 809 DNA (lane 3) also contains a band of 1.06 Kb, the expected size band for the CP4 EPSPS gene. This band contains two expected size CP4 EPSPS genes that are present in

corn line MON 809 (data not shown). The NcoI/EcoRI digests, probed with the CP4 EPSPS gene, identified only the expected size CP4 EPSPS gene.

3. *gox* gene integrity. MON 818 and MON 809 DNAs were digested with NcoI/EcoRI to release the *gox* gene in MON 809 DNA and the Southern blot probed with the *gox* gene. The results are shown in Fig. 5, lanes 1-3. MON 818 DNA was run alone (lane 1) and mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10 also digested with NcoI/EcoRI (lane 2). The MON 818 DNA (lane 1) does not show any bands, as expected for the control DNA. The MON 818 DNA mixed with the plasmids (lane 2) produces a 1.3 Kb band, which corresponds to the expected size of the intact *gox* gene, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). The MON 809 DNA (lane 3) contains one band of 3.5 Kb. The 3.5 Kb band is faint and was observed only with long exposure times. The faintness of the *gox* band suggests that only a part of the *gox* gene is present. The larger than predicted NcoI/EcoRI fragment size (3.5 Kb rather than 1.3 Kb) indicates a DNA rearrangement has occurred within the *gox* gene. Corn line MON 809 appears to contain a partial *gox* gene.

4. Backbone integrity. MON 818 and MON 809 DNAs were digested with NotI to release the intact *nptII/ori-pUC* backbone in MON 809 DNA and the Southern blot probed with the *nptII* gene. The results are shown in Figure 6 (lanes 1 and 2). The digested MON 818 DNA was mixed with 15 pg of PV-ZMBK07 and PV-ZMGT10 also digested with NotI. The MON 818 DNA and plasmid mixture contains two bands of 5.9 Kb and 2.6 Kb (lane 1). The 5.9 Kb band corresponds to the expected size band of the intact backbone from PV-ZMGT10, the 2.6 Kb band corresponds to the expected size band of the intact backbone from PV-ZMBK07 (refer to Fig. 1). The MON 809 DNA contains a 4.2 Kb band (lane 2) which hybridized to the *nptII* probe.

The Southern blot was stripped and reprobed with the *ori-pUC* genetic region. The MON 818 DNA and plasmid mixture (lane 3) contains three bands of 5.9 Kb, 4.2Kb and 2.6 Kb. The 5.9 Kb band corresponds to the expected size band of the intact backbone from PV-ZMGT10, the 2.6 Kb band corresponds to the expected size band of the intact backbone from PV-ZMBK07 (refer to Fig. 1). The 4.2 Kb band is a background band. The MON 809 DNA contains one band, 4.2 Kb in size (lane 4) which corresponds to the band which hybridized to the *nptII* gene in lane 2. The 4.2 Kb background band (lane 3) co-migrates with the one band which hybridized to the *nptII* and *ori-pUC* probes (lane 4). The 4.2 Kb band hybridized to the *nptII* and *ori-pUC* probes, indicating that the backbone is present but is not the predicted size.

III. CONCLUSIONS

The corn line MON 809 was produced by particle acceleration technology with the two plasmids PV-ZMBK07 and PV-ZMGT10 that contained the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes. The I-DNA (23Kb) contains two *cryIA(b)* genes, one which is the correct size, (3.46 Kb), and one which is smaller (less than 1.0 Kb). There are two CP4 EPSPS genes, both of expected size (1.3 Kb). The *gox* gene present in corn line MON 809 is not intact. The *nptII* and *ori-pUC* probings showed that the backbone was present in the corn line MON 809, but was not the predicted size.

Based on these analyses, we conclude that corn line MON 809 contains a single I-DNA with an intact *cryIA(b)* gene and two CP4 EPSPS genes that are responsible for producing the correct size CryIA(b) and CP4 EPSPS proteins.

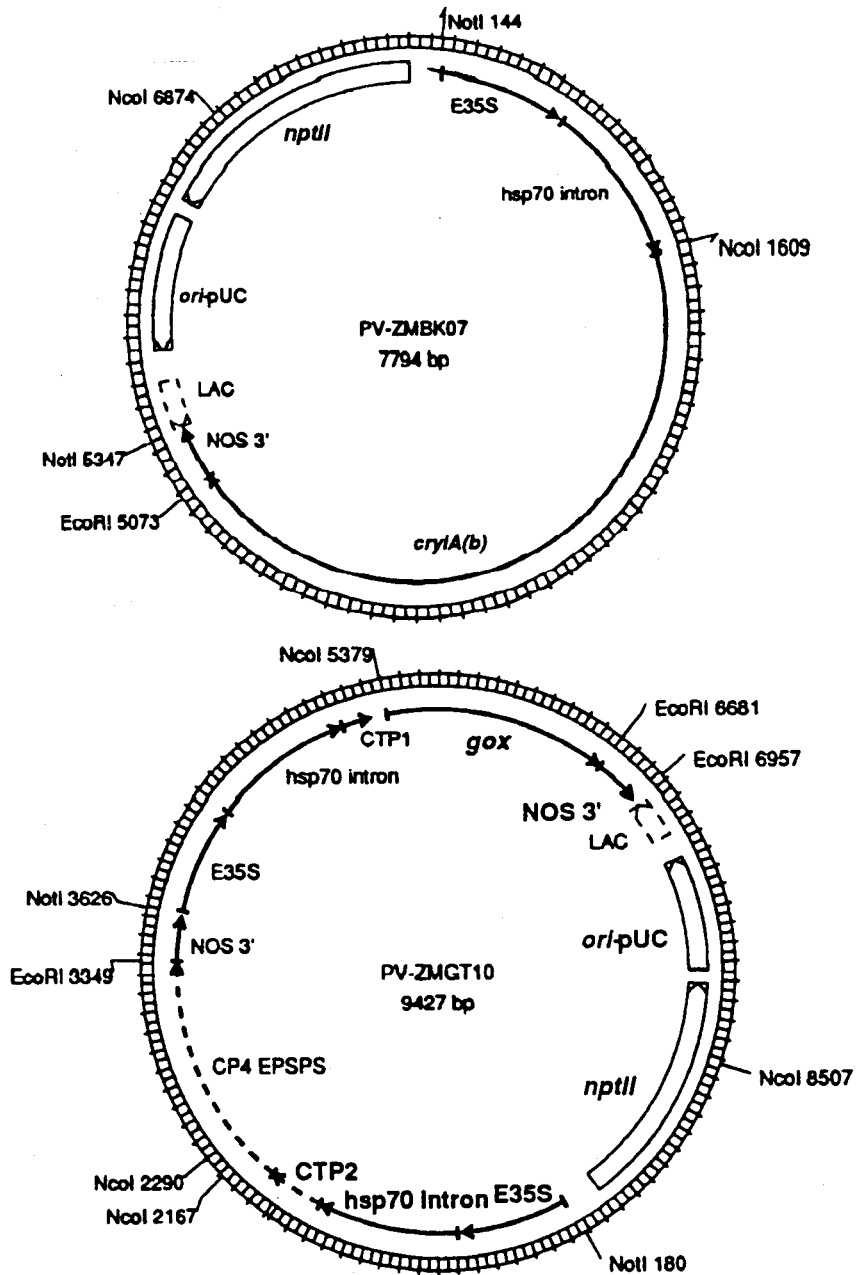


Figure 1. Plasmid maps of PV-ZMBK07 and PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.

Figure 2. Southern blot analysis of corn line MON 809 DNA: insert number analysis

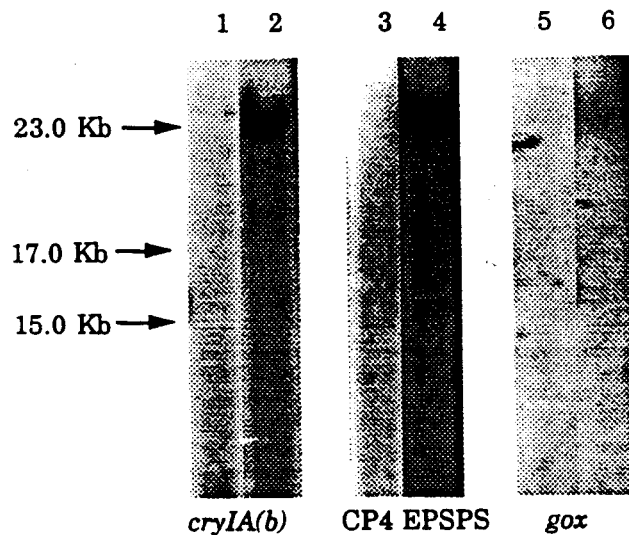


Figure 2. Southern blot analysis of corn line MON 809 DNA. Lanes 1, 3 and 5 contain 12.5 μ g of corn line MON 818 DNA digested with NdeI. Lanes 2, 4 and 6 contain 12.5 μ g of corn line MON 809 DNA digested with NdeI. Lanes 1 and 2 were hybridized with the *cryIA(b)* gene. Lanes 3 and 4 were hybridized with the CP4 EPSPS gene. Lanes 5 and 6 were hybridized with the *gox* gene.

→ Symbol denotes sizes obtained from MW markers.

Figure 3. Southern blot analysis of corn line MON 809 DNA: *cryIA(b)* gene analysis

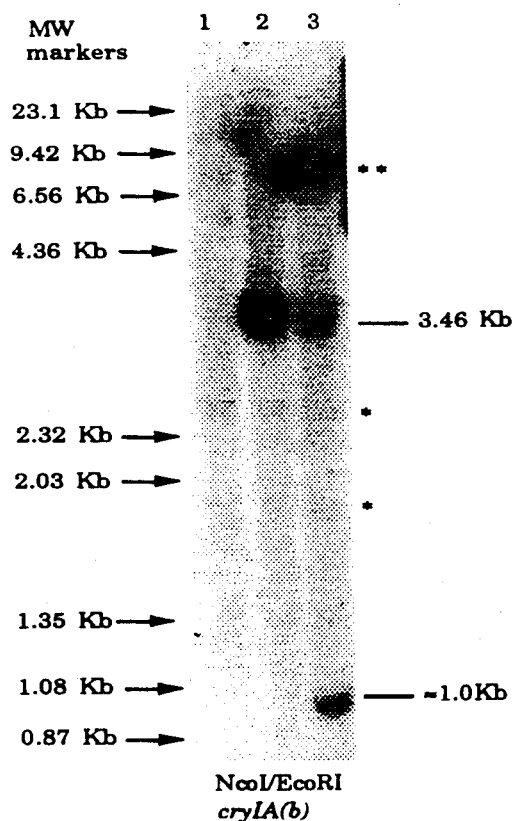


Figure 3. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *cryIA(b)* gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

- Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.
- * Symbol denotes background bands (≈2.5 and 1.9 Kb).
- ~ Symbol denotes a band size approximated from MW marker and plasmid digests.
- ** Symbol denotes an area of non-specific hybridization. This is supported by the observation that the signal is between two lanes.

Figure 4. Southern blot analysis of corn line MON 809 DNA: CP4 EPSPS gene analysis

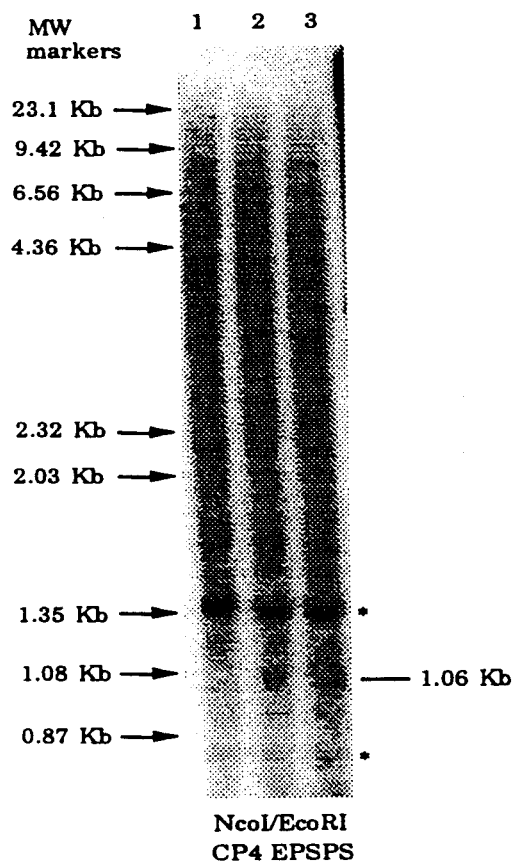


Figure 4. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the CP4 EPSPS gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

- Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.
- * Symbol denotes background bands (≈1.37 and 0.80 Kb).
- Symbol denotes a band size approximated from MW marker and plasmid digests.

Figure 5. Southern blot analysis of corn line MON 809 DNA: *gox* gene analysis

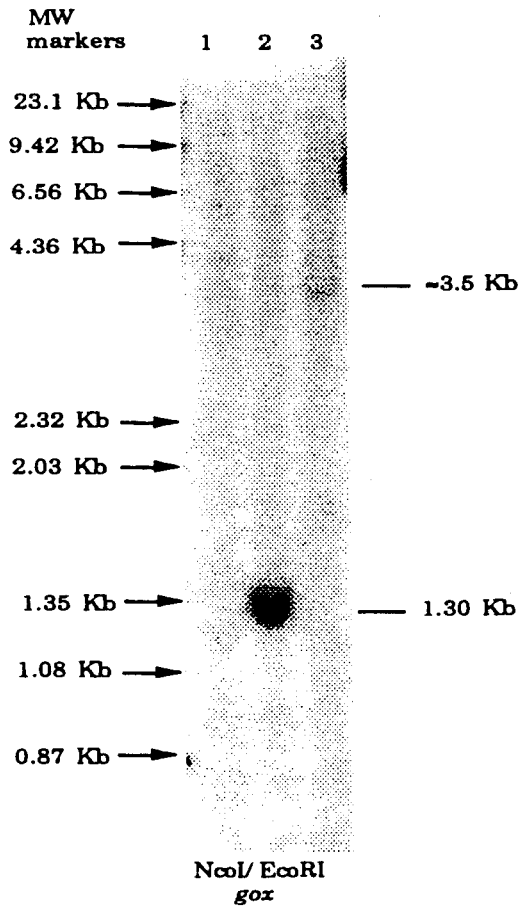


Figure 5. Southern blot analysis of corn line MON 809 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *gox* gene: lane 1, MON 818 DNA; lane 2, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lane 3, MON 809 DNA.

- Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.
- ≈ Symbol denotes a band size approximated from MW marker and plasmid digests.

Figure 6. Southern blot analysis of corn line MON 809 DNA: *nptII* and *ori-pUC* analysis

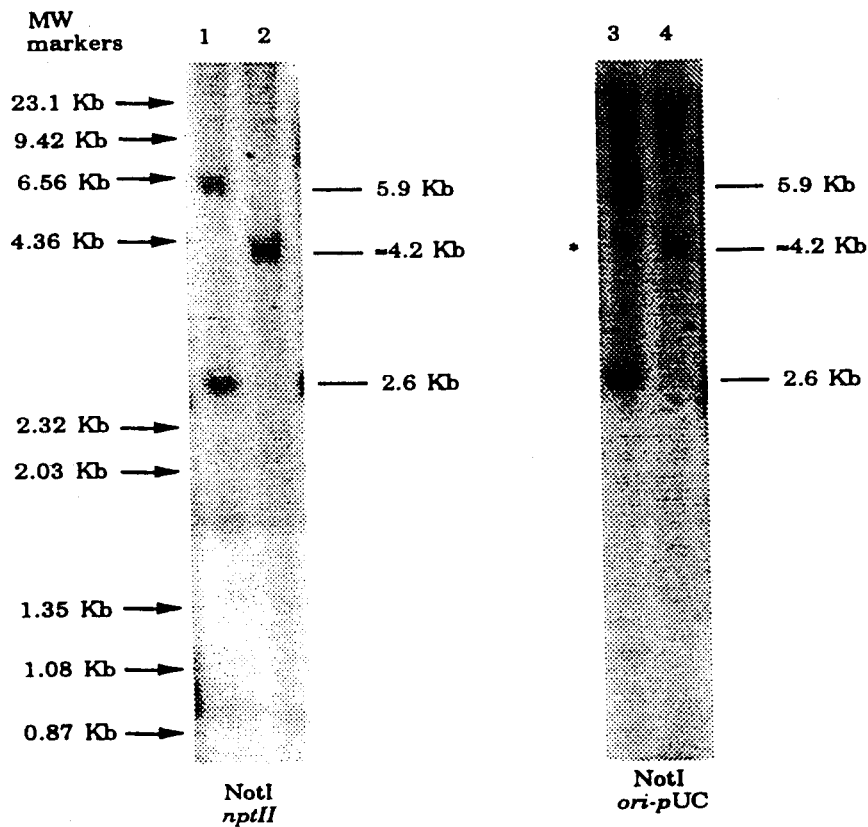


Figure 6. Southern blot analysis of corn line MON 809 DNA. Lanes 1-4 contain the following DNAs digested with NotI: lanes 1 and 3, MON 818 DNA mixed with 15 pg of plasmids PV-ZMBK07 and PV-ZMGT10; lanes 2 and 4, MON 809 DNA. Lanes 1 and 2 were hybridized with the *nptII* region. Lanes 3 and 4 were hybridized with the *ori-pUC* region.

- Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.
- * Symbol denotes background bands.
- Symbol denotes a band size approximated from MW marker and plasmid digests.

Molecular Analysis of Insect Protected Maize Line MON 810

Janice Kania, Pamela Keck, Elaine Levine and Patricia Sanders

I. SUMMARY

This report describes the molecular analysis of the integrated DNA in Insect Protected maize line MON 810. Specifically, the insert number (number of integration sites within the maize genome) and the number and integrity of the inserted genes were determined. Maize line MON 810 was produced by particle acceleration technology using a DNA solution containing two plasmids, PV-ZMBK07 and PV-ZMGT10. The maize transformation vectors used to produce maize line MON 810 contain genes encoding 1) *cryIA(b)* gene; 2) CP4 5-enolpyruvyl-shikimate-3-phosphate synthase (CP4 EPSPS); 3) glyphosate oxidoreductase (*gox*); and 4) the *nptII* gene, under the control of a bacterial-specific promoter. Molecular analysis of maize line MON 810 established that the line only contains the *cryIA(b)* gene from plasmid PV-ZMBK07. The line does not contain the CP4 EPSPS, *gox*, or *nptII* genes. There is no evidence that any of the DNA contained in plasmid PV-ZMGT10 was inserted. Maize line MON 810 contains one integrated DNA, contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, maize hsp70 intron and the *cryIA(b)* gene.

Genetic Element	Maize Line MON 810
<i>cryIA(b)</i> gene	present
CP4 EPSPS gene	not present
<i>gox</i> gene	not present
<i>nptII</i> /ori-pUC	not present

II. RESULTS AND DISCUSSION

A. Southern blot results

Plasmid PV-ZMBK07 contained the *cryIA(b)* gene and plasmid PV-ZMGT10 contained the CP4 EPSPS and *gox* genes. The maps of the two plasmid vectors, along with the locations of the restriction sites utilized for Southern analyses, are presented in Figure 1.

The DNAs from MON 818 and MON 810 plants were digested with a variety of restriction enzymes and subjected to Southern blot hybridization analyses to characterize the DNA that was transferred during the particle acceleration into the maize genome. Specifically, the insert number (number of integration sites within the maize genome), and the copy number and integrity of each gene was examined.

B. Insert Number

NdeI digestion results. The purpose of the NdeI digests was to determine the number of plasmid DNA inserts in the maize line MON 810. The plasmids PV-ZMBK07 and PV-ZMGT10 do not contain a restriction site for NdeI. Thus this enzyme effectively cleaves outside any inserted DNA, releasing a fragment containing the inserted DNA and adjacent genomic DNA. MON 818 control DNA and MON 810 DNA were digested with NdeI and probed with plasmid PV-ZMBK07 DNA. The results are shown in Figure 2. MON 818 DNA (lane 1), produced one very light, diffused band of approximately 21.0 Kb which is a background band since it is present in both the control MON 818 DNA and the MON 810 DNA. MON 810 DNA produced one band, approximately 5.5 Kb in size (lane 2). This result established that insect protected maize line MON 810 contains one fragment of integrated DNA. The size of the inserted DNA plus adjacent genomic DNA up to the NdeI restriction sites is approximately 5.5 Kb.

C. Insert Composition

1. *cryIA(b)* gene integrity. MON 818 and MON 810 DNAs were digested with NcoI/EcoRI to release the *cryIA(b)* gene and the Southern blot probed with the *cryIA(b)* gene. The results are shown in Figure 3, lanes 1-3. The positive hybridization control (lane 1) produced one 3.46 Kb fragment which corresponds to the expected size of the *cryIA(b)* gene (refer to the plasmid maps in Fig. 1). Due to the plasmid DNA not being mixed with genomic control DNA the band appears larger than its true molecular weight. The MON 818 DNA (lane 2) does not produce any bands, as expected for the control line. The MON 810 DNA (lane 3) contains one band, approximately 3.1 Kb.

2. CP4 EPSPS gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMGT10) and insect protected maize line MON 810 DNA were digested with NcoI/BamHI to release the CP4 EPSPS gene and the Southern blot probed with the CP4 EPSPS gene. The results are shown in Figure 4, lanes 1 and 2. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMGT10 DNA (lane 1) produced one band, approximately 3.1 Kb in size, which corresponds to the expected size CP4 EPSPS fragment, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). MON 810 DNA (lane 2) shows no hybridizing fragments to the CP4 EPSPS probe, establishing that insect protected maize line MON 810 does not contain the CP4 EPSPS gene.

3. *gox* gene integrity. Plasmid DNAs (PV-ZMBK07 and PV-ZMGT10) and insect protected maize line MON 810 DNA were digested with NcoI/BamHI to release the *gox* gene and the Southern blot probed with the *gox* gene. The results are shown in Figure 4, lanes 3 and 4. Approximately 50 pg of a mixture of PV-ZMBK07 and PV-ZMGT10 DNA (lane 3) produced one band, a NcoI/NcoI fragment, approximately 3.1 Kb, which corresponds to the expected size *gox* fragment, as predicted from the plasmid map (PV-ZMGT10 in Fig. 1). MON

810 DNA (lane 4) shows no hybridizing fragments to the *gox* probe, establishing that insect protected maize line MON 810 does not contain the *gox* gene.

4. Backbone integrity. Plasmid PV-ZMBK07, control line MON 818 and insect protected maize line MON 810 DNAs were digested with NcoI/EcoRI to release the *nptII/ori-pUC* backbone and the Southern blot probed with the *nptII* gene. The results are shown in Figure 5 (lanes 1-3). Approximately 50 pg of PV-ZMBK07 DNA produced two bands of 2.5 Kb and 1.8 Kb (lane 1). The 2.5 Kb and 1.8 bands correspond to the expected size fragments of the backbone from vector PV-ZMBK07 (refer to Fig. 1). The MON 818 DNA alone (lane 2) does not produce any bands, as expected from a non-modified control line. MON 810 DNA (lane 3) shows no bands, establishing that the backbone sequences were not integrated in insected protected maize line MON 810.

The Southern blot was stripped and reprobed with the *ori-pUC* genetic region. The PV-ZMBK07 and PV-ZMGT10 DNAs (lane 4) contains one band of 1.8 Kb. The 1.8 Kb band corresponds to the expected size fragment of the backbone from PV-ZMBK07 (refer to Fig. 1). The MON 818 DNA alone (lane 5) does not produce any bands, as expected for the unmodified control line. MON 810 DNA (lane 6) shows no bands, establishing that the backbone sequences were not integrated in insected protected maize line MON 810. The lack of observed bands with both *ori-pUC* and *nptII* probes, established that insect protected maize line MON 810 does not contain any backbone sequences.

V. CONCLUSIONS

The insect protected maize line MON 810 was produced by particle acceleration technology with a DNA solution that contained the *cryIA(b)*, CP4 EPSPS, *gox* and *nptII* genes. Maize line MON 810 contains one integrated DNA contained on a 5.5 Kb NdeI fragment, which contains the E35S promoter, maize *hsp70* intron and the *cryIA(b)* gene. Insect protected maize line MON 810 does not contain a CP4 EPSPS gene, a *gox* gene or *nptII/ori-pUC* sequences. The continued efficacy of maize line MON 810 confirms that an insecticidally active CryIA(b) protein is produced which provides season long control of European Corn Borer.

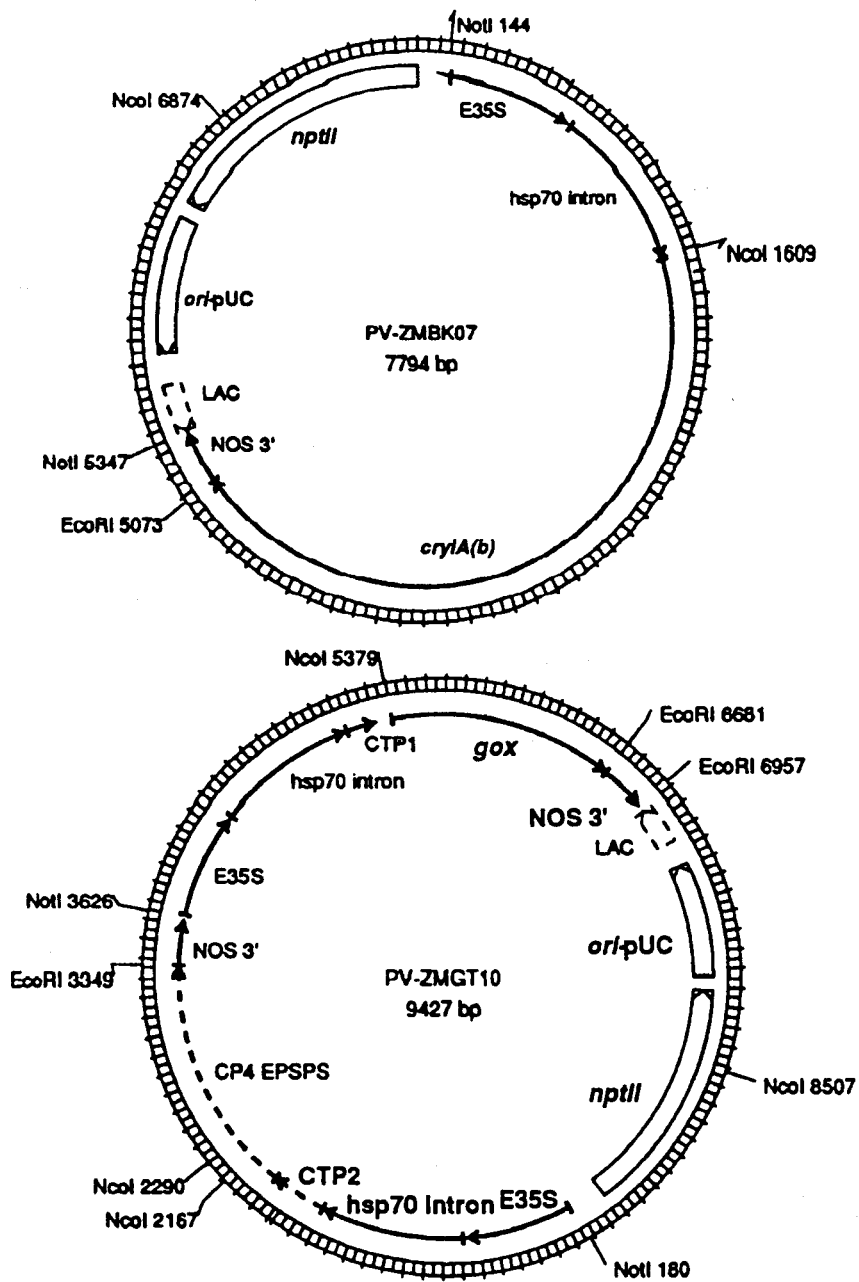


Figure 1. Plasmid maps of PV-ZMBK07 and PV-ZMGT10. Restriction sites, and their locations in base pairs, used during Southern analyses are shown.

Figure 2. Southern blot analysis of maize line MON 810 DNA: insert number analysis

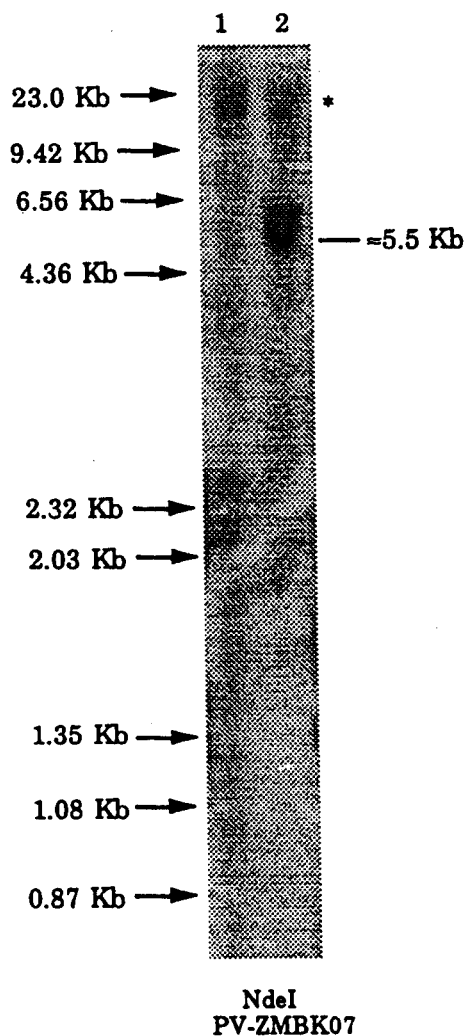


Figure 2. Southern blot analysis of maize line MON 810 DNA. Lanes 1 and 2 contain the following DNAs digested with NdeI and probed with PV-ZMBK07: lane 1, MON 818 DNA; lane 2, MON 810 DNA.

- Symbol denotes sizes obtained from MW markers.
- = Symbol denotes a band size approximated from MW marker and plasmid digests.
- * Symbol denotes background bands.

Figure 3. Southern blot analysis of maize line MON 810 DNA: *cryIA(b)* gene analysis

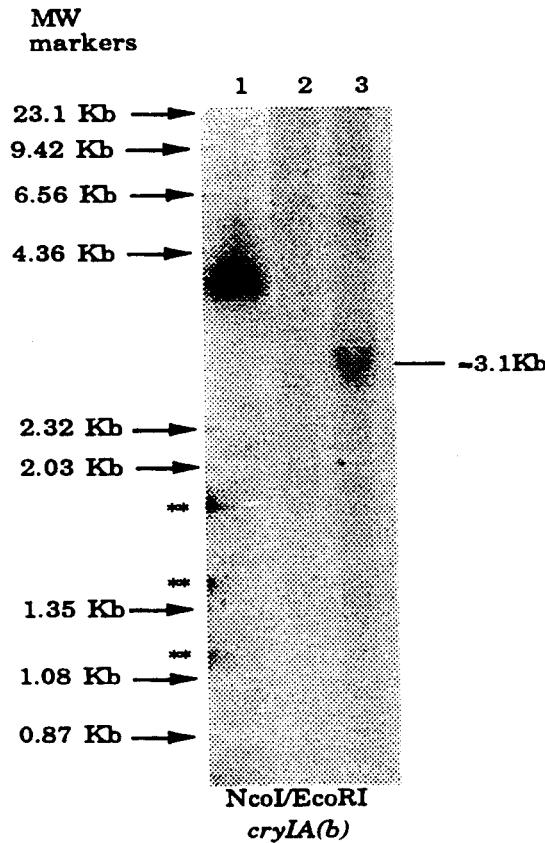


Figure 3. Southern blot analysis of maize line MON 810 DNA. Lanes 1-3 contain the following DNAs digested with NcoI/EcoRI and probed with the *cryIA(b)* gene: lane 1, ≈50 pg of plasmid PV-ZMBK07; lane 2, MON 818 DNA, lane 3, MON 810 DNA.

- Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.
- = Symbol denotes a band size approximated from MW marker and plasmid digests.
- ** Symbol denotes an area of hybridization in an adjacent lane which only appears to be in lane 1, due to the contents of the lanes migrating at an angle in this portion of the gel.

Figure 4. Southern blot analysis of maize line MON 810 DNA: CP4 EPSPS and *gox* gene analysis

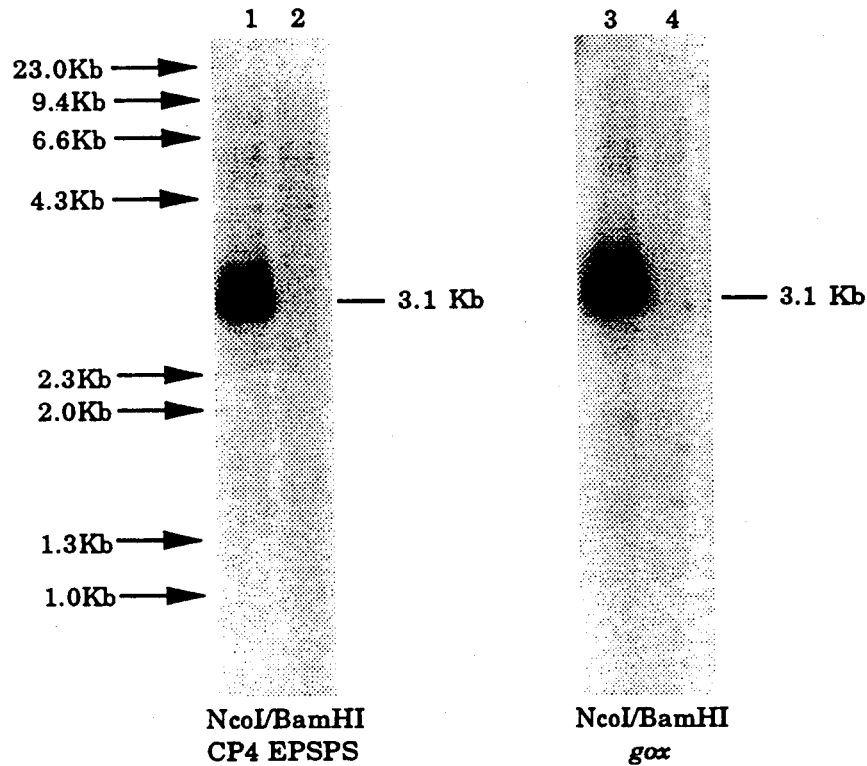


Figure 4. Southern blot analysis of maize line MON 810 DNA. Lanes 1-4 contain the following DNAs digested with NcoI/BamHI: lanes 1 and 3, ~50pg of plasmids PV-ZMGT10 and PV-ZMBK07; lanes 2 and 4, MON 810 DNA. Lanes 1 and 2 were hybridized with the CP4 EPSPS gene. Lanes 3 and 4 were hybridized with the *gox* gene.

- ➔ Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.

Figure 5. Southern blot analysis of maize line MON 810 DNA: *nptII* and *ori-pUC* analysis

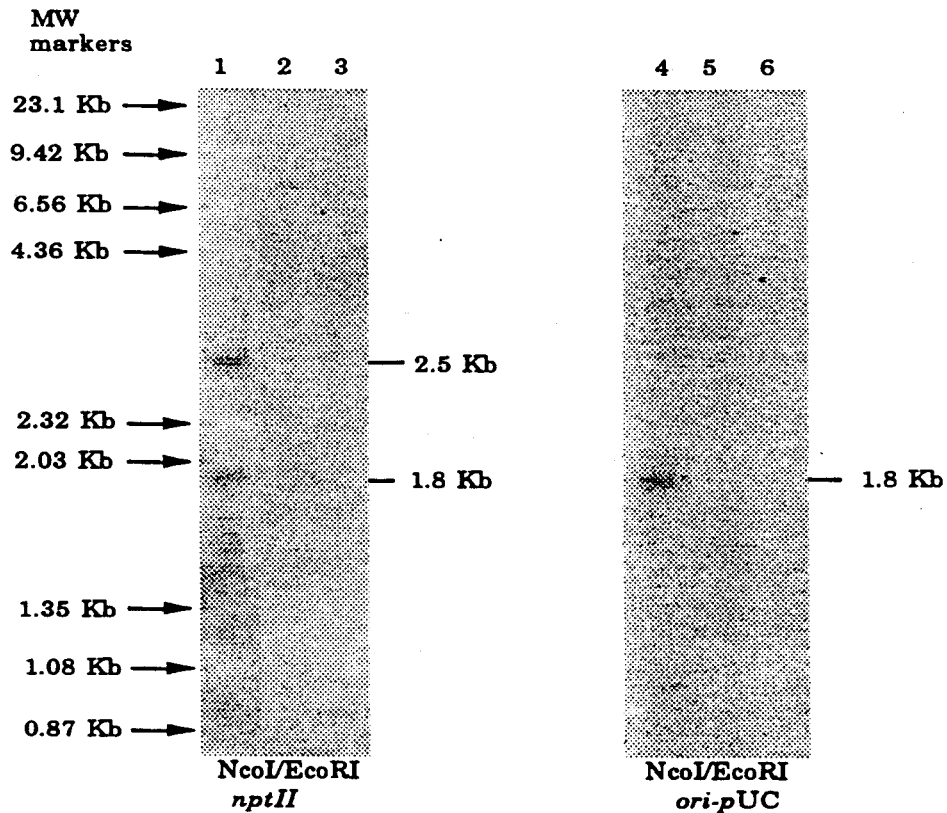


Figure 5. Southern blot analysis of maize line MON 810 DNA. Lanes 1-6 contain the following DNAs digested with *NcoI/EcoRI*: lanes 1 and 4, ≈ 50 pg of plasmid PV-ZMBK07; lanes 2 and 5, MON 818 DNA; lanes 3 and 6, MON 810 DNA. Lanes 1-3 were hybridized with the *nptII* region. Lanes 4-6 were hybridized with the *ori-pUC* region.

- Symbol denotes sizes obtained from MW markers on ethidium stained gel.
- Symbol denotes sizes obtained from plasmid digests.

Monsanto

96-017-01P

Monsanto Company
700 Chesterfield Parkway North
St. Louis, Missouri 63198
Phone: (314) 694-1000

January 8, 1996

Mr. Michael A. Lidsky
Deputy Director, BBEP, APHIS, USDA
6505 Belcrest Road
Federal Building
Hyattsville, MD 20782

Subject: Petition for Determination of Non-Regulated Status: Additional YieldGard™ Corn (*Zea mays* L.) Lines with the cryIA(b) Gene from *Bacillus thuringiensis* subsp. *kurstaki*.
Monsanto #: 95-274U

Dear Mr. Lidsky:

The Agricultural Group of Monsanto Company is submitting a Petition for Determination of Non-Regulated Status to the Animal and Plant Health Inspection Service (APHIS) regarding additional corn lines which express a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). Field experiments were conducted in 1993 and 1994 in the U.S. corn growing region under United States Department of Agriculture (USDA) permits or notifications as well as an Experimental Use Permit (524-EUP-82) obtained from the EPA in 1994 and renewed in 1995. Results from these field experiments have demonstrated that YieldGard corn lines MON 809 and 810 are protected season long from the leaf and stalk feeding damage caused by European corn borer (*Ostrinia nubilalis*).

This petition requests a determination from APHIS that YieldGard™ corn lines MON 809 and 810, any progenies derived from crosses between MON 809 and 810 and traditional corn varieties, and any progeny derived from crosses of MON 809 and 810 with transgenic corn varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under regulations in 7 CFR part 340. These two additional corn lines were originally identified in USDA Petition 95-093-01p for YieldGard corn line MON 80100 submitted to the agency on March 30, 1995 and approved August 22, 1995 (FR 60:171; pp. 46107-46108).

1-11-96
2

We appreciate your attention to this matter. Should you have any questions, please feel free to contact either Dr. Dickerson at 202-383-2857 or myself (314-537-7488).

Sincerely,

A handwritten signature in cursive script, appearing to read "Kent A. Croon".

Kent A. Croon, Ph.D.
Regulatory Affairs Manager

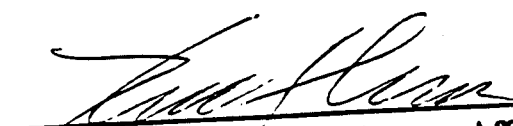
cc: Dr. C.T. Dickerson - Monsanto

Petition for Determination of Nonregulated Status:

Additional YieldGard™ Corn (*Zea mays* L.) Lines with the cryIA(b)
Gene from *Bacillus thuringiensis* subsp. *kurstaki*

The undersigned submits this petition of 7 CFR 340.6 to request that
the Director, BBEP, make a determination that additional lines of
YieldGard™ corn should not be a regulated article
under 7 CFR part 340.

Submitted by:



Kent A. Croon, Regulatory Affairs Manager
Ceregen, a Unit of Monsanto Company, BB3A
700 Chesterfield Parkway North
Chesterfield, MO 63198
Tel: 314-537-7488
Fax: 314-537-7085

January 8, 1996
#95-274U

Prepared by:

K.A. Croon, P.R. Sanders, J. Kania, P. Keck, E. Levine,
and G.B. Parker

**Additional YieldGard™ Corn (*Zea mays* L.) Lines with the cryIA(b)
Gene from *Bacillus thuringiensis* subsp. *kurstaki***

Summary

Monsanto Company is submitting this Petition for Determination of Non-regulated Status to the Animal Plant Health Inspection Service (APHIS) regarding additional corn lines which express a CryIA(b) protein derived from the common soil bacterium *Bacillus thuringiensis* subsp. *kurstaki* (*B.t.k.*). This petition requests a determination from APHIS that YieldGard™ corn lines MON 809 and 810, any progenies derived from crosses between MON 809 and 810 and traditional corn varieties, and any progeny derived from crosses of MON 809 and 810 with transgenic corn varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under regulations in 7 CFR part 340. These two additional corn lines were originally identified in USDA Petition 95-093-01p for YieldGard corn line MON 80100 submitted to the agency on March 30, 1995 and approved August 22, 1995 (FR 60:171; pp. 46107-46108).

Field experiments were conducted in 1993 and 1994 in the U.S. corn growing region under United States Department of Agriculture (USDA) permits or notifications as well as an Experimental Use Permit (524-EUP-82) obtained from the EPA in 1994 and renewed in 1995. Results from these field experiments have demonstrated that YieldGard corn lines MON 809 and 810 are protected season long from the leaf and stalk feeding damage caused by European corn borer (*Ostrinia nubilalis*). Growers planting YieldGard corn will not require insecticide applications to control European corn borer (ECB). This reduction in insecticide use will enhance biological control and the implementation of other pest management strategies for other corn pests. In addition, these plants exhibit no pathogenic properties, are no more likely to become weeds than the non-modified parental corn lines, are unlikely to increase the weediness potential for any other cultivated plants or native species, and are equivalent morphologically, agronomically, and compositionally to the parental corn lines.

The use of YieldGard corn will have a more positive impact on the environment than the use of chemical insecticides to control ECB. The CryIA(b) protein is ecologically benign, i.e., it breaks down rapidly in the soil,

YieldGard™ is a registered trademark of Monsanto Company, St. Louis, MO.

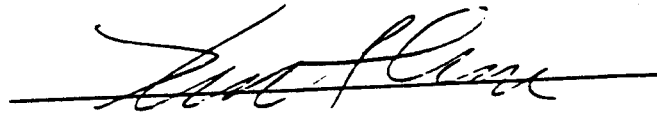
and is safe to non-target organisms such as fish, birds, mammals, and beneficial insects. In addition, the risk of an uncontrolled introduction of this corn into the environment through hybridization or outcrossing to native species is virtually non-existent in the U.S.

In conclusion, the consistent control afforded by YieldGard corn lines MON 809 and 810 will enable growers to significantly reduce the amount of chemical insecticide now applied to their crop for control of ECB while maintaining yield potential. As a result, they will be able to utilize IPM practices that cannot presently be implemented because of the lack of options other than use of chemical insecticides to control this pest. An increase in the biological and cultural control of non-target corn pests and a more judicious use of chemical insecticides will result in a positive impact on the environment, which will ultimately be advantageous to the grower and the public as well.

Therefore, Monsanto Company requests a determination from APHIS that YieldGard corn lines MON 809 and 810 and any progenies derived from crosses between MON 809 and 810 and traditional corn varieties no longer be considered regulated articles under regulations in 7 CFR part 340.

Certification

The undersigned certifies, that to the best knowledge and belief of the undersigned, this petition includes all information and views on which to base a determination, and that it includes relevant data and information known to the petitioner which are unfavorable to the petition.



Kent A. Croon, Regulatory Affairs Manager
Ceregen, a Unit of Monsanto Company, BB3A
700 Chesterfield Parkway North
Chesterfield, MO 63198
Tel: 314-537-7488
Fax: 314-537-7085

Abbreviations Used in this Petition for the Determination of Non-Regulated Status of Additional YieldGard Corn Lines MON 809 and 810

APHIS	Animal Plant Health Inspection Service
bp, Kb	Base pairs, kilobase pairs
<i>B.t.k.</i>	<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>
CaMV	Cauliflower mosaic virus
CFR	Code of federal regulations
CP4 EPSPS	EPSPS from <i>Agrobacterium</i> sp. strain CP4
<i>cryIA(b)</i>	Gene for class I (Lepidoptera-specific) crystal protein
CryIA(b)	Class I (Lepidoptera-specific) crystal protein
CTP	Chloroplast transit peptide
DNA	Deoxyribonucleic Acid
<i>E. coli</i>	<i>Escherichia coli</i>
E35S	35S promoter with enhancer sequence
ECB	European corn borer
ELISA	Enzyme-linked immunosorbent assay
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FFDCA	Federal Food Drug and Cosmetic Act
FIFRA	Federal Insecticide Fungicide and Rodenticide Act
GLP	Good Laboratory Practice
<i>gox</i>	Gene for glyphosate oxidase
GOX	Glyphosate oxidase protein
hsp70	Intron sequence from heat-shock protein 70
I-DNA	Integrated DNA
IPM	Integrated Pest Management
NOS 3'	3' transcriptional termination sequence from nopaline synthase
NPTII	Neomycin phosphotransferase II
<i>nptII</i>	Gene for neomycin phosphotransferase II
<i>ori-pUC</i>	Bacterial origin of replication from the pUC plasmid
sp	Species
subsp.	Subspecies
USDA	United States Department of Agriculture
µg, g	Microgram, gram

**PETITION FOR DETERMINATION OF NON-REGULATED STATUS
OF ADDITIONAL YIELDGARD CORN LINES MON 809 AND 810**

TABLE OF CONTENTS

	<u>Page</u>
Title Page	1
Summary	2
Certification	4
Abbreviations	5
Table of Contents	6
I. Rationale for Development of YieldGard™ Corn	9
A. Need and Benefits of YieldGard Corn	9
B. Regulatory Approvals	11
C. References	12
II. The Corn Family	13
A. Summary	13
B. References	14
III. Description of the Transformation System and Plasmids Utilized	15
A. Construction of the Plasmid Vectors, PV-ZMBK07 and PV-ZMGT10, Utilized for Transformation	15
B. References	20
IV. Molecular Biology of YieldGard Corn Lines MON 809 and 810	22
Introduction	22
A. Molecular Analysis of YieldGard Corn Line MON 809	22
B. Molecular Analysis of YieldGard Corn Line MON 810	23
C. Segregation Data and Stability of Gene Transfer	24
1. YieldGard Corn Line MON 809	24
2. YieldGard Corn Line MON 810	25
D. Conclusion	26

TABLE OF CONTENTS (continued)

	<u>Page</u>
V. Detailed Description of the Phenotype of YieldGard Corn Lines MON 809 and 810	28
Introduction	28
A. Expression Levels of the CryIA(b), CP4 EPSPS, GOX and NPTII Proteins	29
B. Field Germination Results	30
C. Disease and Pest Susceptibilities	30
D. Yield Characteristics	31
E. Composition Analysis of YieldGard Corn Lines MON 809 and 810	32
F. References	38
VI. Statement of Grounds Unfavorable	39
List of Figures	
Figure III.1 Plasmid map of PV-ZMBK07	16
Figure III.2 Plasmid map of PV-ZMGT10	17
List of Tables	
Table III.1 Summary of DNA elements in the plasmid PV-ZMBK07	18
Table III.2 Summary of DNA elements in the plasmid PV-ZMGT10	19
Table IV.1 Segregation data and analysis of progeny of YieldGard corn line MON 809	24
Table IV.2 Stability of gene transfer based on segregation data for backcross derivatives of YieldGard corn line MON 809 in two unrelated inbred lines (B73 and Mo17)	25
Table IV.3 Segregation data and analysis of progeny of YieldGard corn line MON 810	25

TABLE OF CONTENTS (continued)

	<u>Page</u>	
Table IV.4	Stability of gene transfer based on segregation data for backcross derivatives of YieldGard corn line MON 810 in two unrelated inbred lines (B73 and Mo17)	26
Table V.1	Summary of specific protein levels measured in tissues of YieldGard corn lines MON 809 and 810	29
Table V.2	Field germination results for YieldGard corn lines MON 809 and 810 and control	30
Table V.3	Yield comparison (bushels/acre) of nontransgenic and MON 809 and 810 versions of the same hybrid	31
Table V.4	Summary of proximate analysis of grain from corn lines MON 809, MON 810, and 818 (control).	32
Table V.5	Disease and insect susceptibility of YieldGard corn lines MON 809 in comparison to non-modified corn plants	33
Table V.6	Disease and insect susceptibility of YieldGard corn lines MON 810 in comparison to non-modified corn plants	36

Part I. Rationale for Development of YieldGard™ Corn

A. Need and Benefits of Yieldgard Corn

Corn is the largest U.S. crop in terms of acreage, total production, and crop value (National Corn Growers Association, 1994). European corn borer (ECB) (*Ostrinia nubilalis*) is among the most important corn insect pests in the U.S. and worldwide (Dicke and Guthrie, 1988). This pest ranges from the Eastern seashore west to the Rocky Mountains and from southern Canada to Florida and the Gulf States. In the central corn belt, the pest typically completes two generations each year, but in warm years may complete a partial to full third generation (USDA, 1992). Physical damage results from ECB as a result of: (1) leaf feeding (from the first generation), (2) stalk tunneling (from the first and second generation), (3) leaf sheath and collar feeding (from the second and third generation) and (4) ear damage (from the second and third generation) (USDA, 1992). Researchers from across the pest's geographic range have estimated a five to ten percent corn yield loss annually, attributable to ECB damage (USDA Petition 95-093-01p; Bode and Calvin, 1990; Guthrie *et al.*, 1975; Rice, 1994a-c). Yield losses are attributed to disruption of nutrient and water translocation to key tissues, secondary disease infections, stalk lodging, ear droppage and kernel damage.

Control of ECB using conventional insecticide applications is variable due to difficulties in the proper timing of the application and placement of the insecticide where ECB larvae are feeding. Small deviations from the optimal date for applying an insecticide can result in significantly less control. More than one insecticide application may be necessary. To time these insecticide applications properly, a field scouting program is required (USDA, 1992; USDA Petition 95-093-01p). Hybrids with resistance to the first generation (leaf-feeding resistance) of ECB, obtained through traditional breeding techniques, can reduce the amount of loss. However, to date, these hybrids do not have the yield potential of susceptible full-season hybrids (USDA, 1992).

Monsanto has developed genetically modified corn plants (YieldGard™) that control ECB. This YieldGard corn offers a new mechanism to produce and deliver a highly effective insecticide to target pests (e.g. production by cells of the crop plant rather than industrially and application by spray equipment). The technology couples the environmental advantages of host plant resistance with the efficacy of CryIA(b), an effective biological

insecticide. YieldGard corn expresses the CryIA(b) protein which is selective against certain lepidopteran insects that must feed upon the plants to be controlled. Therefore, this technology offers selective activity without disrupting pest suppression by natural enemies, such as parasites and predators.

The determination that YieldGard corn lines MON 809 and 810 and their progenies are no longer regulated articles and their subsequent commercialization will represent an efficacious and environmentally compatible addition to the existing options for corn insect pest management. The use of YieldGard corn will provide potential benefits to growers, the general public and the environment, including:

- A more reliable, economical, and less labor intensive means to control ECB.
- Insect control without harming non-target species, including humans.
- A means for growers to significantly reduce the amount of chemical insecticides now applied to the crop thereby achieving ECB control in a more environmentally compatible manner than is currently available.
- A reduction in the manufacturing, shipment, and storage of chemical insecticides used in corn.
- A reduction in the exposure to workers to the pesticide and pesticide spray solution.
- A reduction in the number of empty pesticide containers and amount of pesticide spray solution that must be disposed of according to applicable environmental regulations.
- An ideal fit with Integrated Pest Management (IPM) and sustainable agricultural systems.
- Both large and small growers will benefit from the planting of YieldGard corn as no additional labor, planning, or machinery is required.

B. Regulatory Approvals

Before commercializing YieldGard corn lines MON 809 and 810, Monsanto will seek the following regulatory approvals:

1. This determination from USDA/APHIS that YieldGard corn lines MON 809 and 810, and all progenies from crosses between YieldGard corn lines MON 809 and 810 and other corn varieties, are no longer a regulated article according to 7CFR §340.6.
2. Regulatory approval from the Environmental Protection Agency (EPA) of the CryIA(b) insecticidal protein as expressed in YieldGard corn under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). This petition has been submitted.
3. An exemption from the requirement of a tolerance for the CryIA(b) insecticidal protein, the CP4 EPSPS selectable marker enzyme, and the genetic material necessary for the production of these proteins in or on all agricultural commodities under sections 408 of the Federal Food Drug and Cosmetic Act (FFDCA) from the EPA.

In addition, we will complete our consultations which have been initiated with the FDA under their May 29, 1992 policy statement concerning foods derived from new plant varieties.

Monsanto will consult with the pesticide and, if applicable, biotechnology regulatory officials of the states in which the commercial product will be sold and obtain a state license, if such is required.

C. References

- Bode, W.M. and D.D. Calvin. 1990. Yield-loss relationships and economic injury levels for European corn borer (Lepidoptera: Pyralidae) populations infesting Pennsylvania field corn. *J. Econ. Entomology*. 83(4): 1595-1603.
- Dicke, F.F. and W.D. Guthrie. 1988. The most important corn insects. pp 767 -867. *In* Spraugue, G.F. and Dudley, J.W., Editors. *Corn and Corn Improvement, Third Edition*. Number 18 in the series *Agronomy*. American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., Wisconsin, USA.
- Guthrie, W.D., W.A. Russell, F.L. Neumann, G.L. Reed, and R.L. Grindeland. 1975. Yield losses in maize caused by different levels of infestation of second-brood European corn borers. *Iowa State Journal of Research*. Vol. 50, no. 2.
- National Corn Growers Association. 1994. *The World of Corn*. St. Louis, Missouri, USA.
- Rice, M.E. 1994a. Aerial application of insecticides for second generation European corn borer control 1993. p. 206. *In* *Arthropod Management Tests: 1994*. Entomological Society of America, Lanham, Maryland.
- Rice, M.E. 1994b. Second generation European corn borer control with aerial application of insecticides. 1992. pp. 205-206. *In* *Arthropod Management Tests: 1994*. Entomological Society of America, Lanham, Maryland.
- Rice, M.E. 1994c. Aerial application of insecticides for control of second generation European corn borer. 1991. p. 204-205. *In* *Arthropod Management Tests: 1994*. Entomological Society of America, Lanham, Maryland.
- USDA. 1992. *European Corn Borer - Development and Management*. North Central Regional Extension Publication No. 327. Printing and Publications, Iowa State University, Ames, IA.
- USDA Petition 95-093-01p. Insect Protected Corn (*Zea mays* L.) with the *cryIA(b)* Gene from *Bacillus thuringiensis* subsp. *kurstaki*. FR 60:171 pp. 46107-46108.

Part II. The Corn Family

A. Summary

Corn (*Zea mays* L.), or maize, is one of the few major crop species indigenous to the Western Hemisphere (Goodman, 1988). Corn is grown in nearly all areas of the world and ranks third behind rice (*Oryza sativa* L.) and wheat (*Triticum* sp.) in total production. Corn has been studied extensively, and it seems the probable domestication of corn was in southern Mexico more than 7,000 - 10,000 years ago (Gould, 1968; Galinat, 1988; Jungenheimer, 1976). The putative parents of corn have not been recovered, but it seems teosinte probably played an important role in the genetic background of corn (Mangelsdorf, 1974).

The transformation from a wild, weedy species to one dependent on humans for its survival probably evolved over a long period of time by the indigenous inhabitants of the Western Hemisphere. Corn, as we know it today, cannot survive in the wild, because the female inflorescence (the ear) restricts seed dispersal (Galinat, 1988; Goodman, 1988; Mangelsdorf, 1986; Wilkes, 1986). Although grown extensively throughout the world, corn is not considered a persistent weed nor one difficult to control.

A summary of the history, taxonomy, genetics, life cycle, and potential gene flow of corn is located in USDA petition 95-093-01p as prepared by Dr. Arnel R. Hallauer, Department of Agronomy, Iowa State University, Ames, Iowa.

B. References

- Galinat, Walton C. 1988. The origin of corn. p. 1-31. *In*: G. F. Sprague and J. W. Dudley (ed.) *Corn and Corn Improvement*. 3rd ed. American Society of Agronomy. Madison, WI.
- Goodman, M.M. 1988. The History and Evolution of Maize. *CRC Critical Rev. Plant Sciences* 7:197-220. CRC Press, Boca Raton, FL.
- Gould, F.W. 1968. *Grass Systematics*. McGraw Hill, New York, NY
- Jungenheimer, R.W. 1976. *Corn Improvement, Seed Production, and Uses*. Jown Wiley & Sons, Inc. New York, NY.
- Mangelsdorf, P.C. 1974. *Corn - Its Origin, Evolution, and Improvement*. Harvard Univ. Press. Cambridge, MA.
- Mangelsdorf, P.C. 1986. The origin of maize. *Scientific American* 255(2):80-86.
- Wilkes, H.G. 1986. Maize: Domestication, racial evolution and spread. *World Archaeological Congress XI*, London.

Part III. Description of the Transformation System and Plasmids Utilized

A. Construction of the Plasmid Vectors, PV-ZMBK07 and PV-ZMGT10, Utilized for Transformation

YieldGard corn lines MON 809 and 810 were produced with a DNA solution containing two plasmid vectors, PV-ZMBK07 and PV-ZMGT10. Plasmid DNA was introduced into the plant tissue using the particle acceleration method as previously identified (USDA Petition 95-093-01p). The PV-ZMBK07 plasmid contains the *cryIA(b)* gene and PV-ZMGT10 contains the CP4 EPSPS and *gox* genes. Both plasmids contain the *nptII* gene under the control of a bacterial promoter and an origin of replication from a pUC plasmid, required for selection and replication in bacteria, respectively. The plasmid vector PV-ZMBK07 is shown in Figure III.1 and PV-ZMGT10 is shown in Figure III.2. A description of the DNA elements in PV-ZMBK07 and PV-ZMGT10 are provided in Tables III.1 and III.2, respectively.

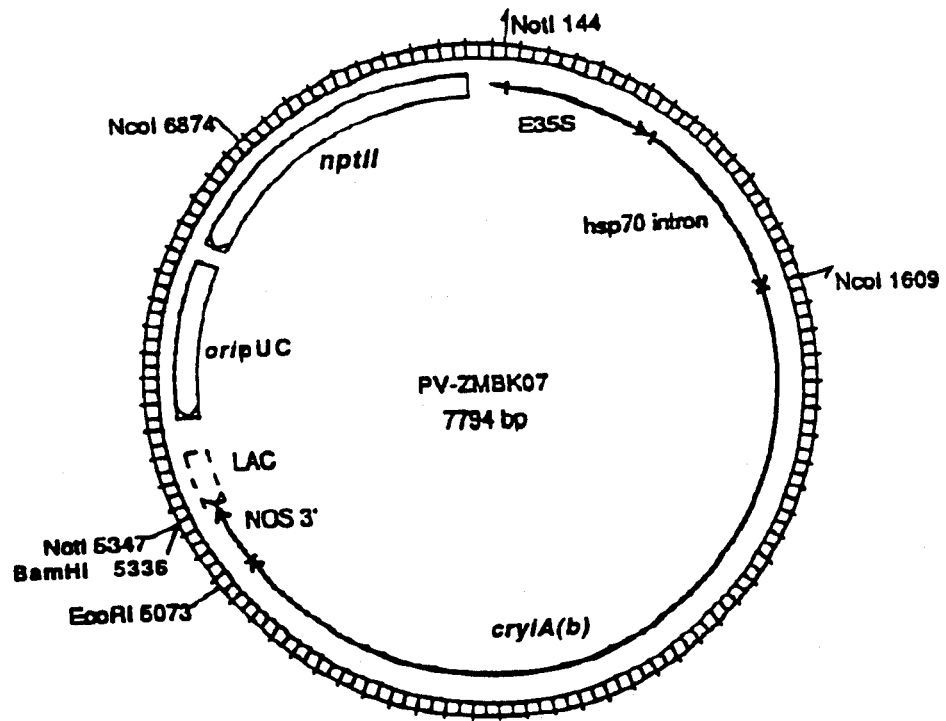


Figure III.1 Plasmid map of PV-ZMBK07.

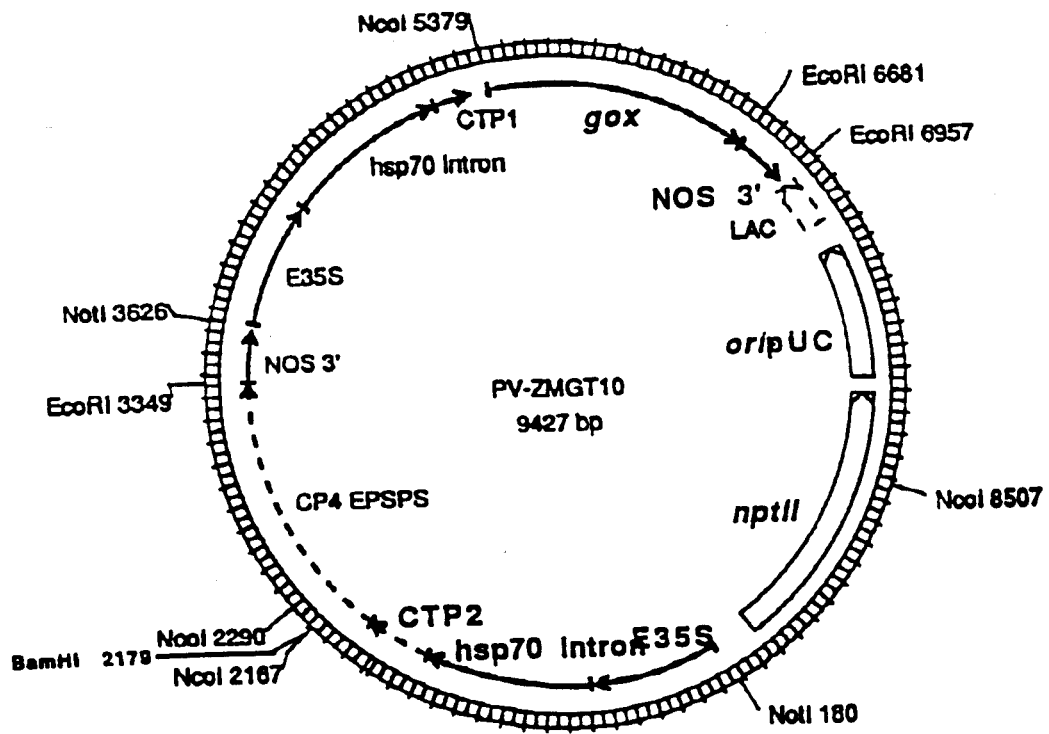


Figure III.2 Plasmid map of PV-ZMGT10.

Table III.1 Summary of DNA elements in the plasmid PV-ZMBK07

Genetic Element	Size, Kb	Function
E35S	0.61	The cauliflower mosaic virus (CaMV) promoter (Odell <i>et al.</i> , 1985) with the duplicated enhancer region (Kay <i>et al.</i> , 1985).
<i>hsp70</i> intron	0.80	Intron from the maize <i>hsp70</i> gene (heat-shock protein) present to increase the level of gene transcription (Rochester <i>et al.</i> , 1986).
<i>cryIA(b)</i>	3.46	The gene encodes the nature identical CryIA(b) protein product (Fischhoff <i>et al.</i> , 1987).
NOS 3'	0.26	A 3' nontranslated region of the nopaline synthase gene which terminates transcription and directs polyadenylation (Fraley <i>et al.</i> , 1983).
<i>lacZ</i>	0.24	A partial <i>E. coli lacI</i> coding sequence, the promoter Plac, and a partial coding sequence for beta-D-galactosidase or <i>lacZ</i> protein from pUC119 (Yanisch-Perron <i>et al.</i> , 1985).
<i>ori-pUC</i>	0.65	The origin of replication for the pUC plasmids that allows for plasmid replication in <i>E. coli</i> (Vieira and Messing, 1987).
<i>nptII</i>	0.79	The gene for the enzyme neomycin phosphotransferase type II. This enzyme confers resistance to aminoglycoside antibiotics and thereby allows for selection of bacteria containing the plasmid (Beck <i>et al.</i> , 1982).