Event 5307 Maize:

Insert Sequence Analysis

AMENDED REPORT NO.1

Data Requirement:	Not applicable
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Company: *Syngenta Seeds, Inc.*

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Demetra Vlachos — November 2, 2010

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Date

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This study was not conducted in compliance with the relevant provisions of Good Laboratory Practices Standards (GLPS) (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act. However, all components of the study were performed according to accepted scientific practices, and relevant study records (including raw data) have been retained.

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LIST OF ACRONYMS AND ABBREVIATIONS

3′	three prime
5′	five prime
aadA	streptomycin adenylyltransferase gene from *Escherichia coli* that confers resistance to streptomycin and spectinomycin
BC	backcross
bp	base pair
CMP	cestrum yellow leaf curling virus promoter
ColE1 ori	*Escherichia coli* origin of replication 1
Cry	crystal protein
cry1Ab	Cry1Ab gene
Cry1Ab	Cry1Ab protein
cry3A	Cry3A gene
Cry3A	Cry3A protein
CTAB	cetyltrimethyl ammonium bromide
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
ecry3.1Ab	eCry3.1Ab gene
eCry3.1Ab	eCry3.1Ab protein
EDTA	ethylenediaminetetraacetic acid
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
g	gram
GLPS	Good Laboratory Practices Standards
LB	left border
M	molar
manA	phosphomannose isomerase gene
mcry3A	modified Cry3A gene
mCry3A	modified Cry3A protein
ml	milliliter
mM	millimolar
NaCl	sodium chloride
NOS	nopaline synthase
NTI	New Technologies Informax
PCR	polymerase chain reaction
pmi	phosphomannose isomerase gene
PMI	phosphomannose isomerase protein
RB	right border
repA	pVS1 replication gene from *Pseudomonas aeruginosa*
spec	streptomycin adenylyltransferase gene from *Escherichia coli*
T_0	original transformant
T-DNA	transferred deoxyribonucleic acid
TE	tris-EDTA
Tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
US EPA	United States Environmental Protection Agency
v/v	volume to volume
vir	virulence regulon in *Agrobacterium tumefaciens*

LIST OF ACRONYMS AND ABBREVIATIONS *(Continued)*

virG	part of the two-component regulatory system for the virulence regulon in *Agrobacterium tumefaciens*
VirGN54D	VirG protein with a N54D substitution
VS1 ori	plasmid pVS1 origin of replication and partitioning region
w/v	weight to volume
ZmUbiInt	*Zea mays* ubiquitin promoter with intron
°C	degrees Celsius
®	registered trademark
™	trademark
µg	microgram
×	cross
× *g*	times gravity
⊗	self-pollination

REPORT AMENDMENTS

Amendment No. 1: November 3, 2010

This amended report has the following corrections:

On page 1, the department title has been changed to Product Safety.

On page 2, the Regulatory Affairs Manager name has been updated.

On page 3, the Regulatory Affairs Manager and Sponsor names have been updated, and position and department titles for the Study Director and the Sponsor have been updated.

On page 4, the Table of Contents has been updated.

On page 8, a new section has been added listing the Report Amendments.

On page 10, a reference citation was added.

On page 10, the Accession number for the gene *ecry3.1Ab* has been updated.

On page 11, typographical errors in the description of *ecry3.1Ab* have been corrected.

On page 21, the Sponsor name has been updated, and position and department titles for the Study Director and the Sponsor have been updated.

On page 24, an additional reference was added.

The corrected pages in this amended report SSB-159-10 A1 are indicated as "*REVISED*".

SUMMARY

Using the techniques of modern molecular biology, Syngenta has transformed maize (*Zea mays*) to produce Event 5307 maize, a new cultivar that has insecticidal activity against certain corn rootworm (*Diabrotica*) species. Maize plants derived from transformation Event 5307 ("5307 maize") contain the gene *ecry3.1Ab* encoding an eCry3.1Ab protein and the gene *pmi* (also known as *manA*) encoding the enzyme phosphomannose isomerase (PMI).

The purpose of this study is to determine the deoxyribonucleic acid (DNA) sequence of the 5307 maize insert and to assess the intactness of the insert, the organization of the functional elements, and the presence of any rearrangements, deletions, and/or base pair changes within the 5307 maize insert.

Two overlapping fragments that span the 5307 maize insert were amplified from genomic DNA extracted from 5307 maize using polymerase chain reaction. These fragments were cloned, and sequences of the clones were aligned to create a consensus of the transferred-DNA (T-DNA) sequence. This sequence was compared to plasmid pSYN12274, the transformation plasmid used to create 5307 maize.

The DNA sequence analysis demonstrated that the 5307 maize insert was intact and that the organization of the functional elements within the insert, as present in plasmid pSYN12274, was maintained.

INTRODUCTION

Using the techniques of modern molecular biology, Syngenta has transformed maize (*Zea mays*) to produce Event 5307 maize, a new cultivar that has insecticidal activity against certain corn rootworm (*Diabrotica*) species. Maize plants derived from transformation Event 5307 ("5307 maize") contain the gene *ecry3.1Ab* encoding an eCry3.1Ab protein and the gene *pmi* (also known as *manA*) encoding the enzyme phosphomannose isomerase (PMI). The eCry3.1Ab protein is an engineered chimera of modified Cry3A (mCry3A) and Cry1Ab proteins (Walters *et al.* 2010). The gene *pmi* was obtained from *Escherichia coli* strain K-12 and the protein it encodes was utilized as a plant selectable marker during development of 5307 maize.

The purpose of this study is to determine the deoxyribonucleic acid (DNA) sequence of the 5307 maize insert and to assess the intactness of the insert, the organization of the functional elements, and the presence of any rearrangements, deletions, and/or base pair changes within the 5307 maize insert. The insert sequence was compared to plasmid pSYN12274, the transformation plasmid used to create 5307 maize.

The DNA sequence analysis comparing the 5307 maize insert sequence and the plasmid pSYN12274 sequence demonstrated that the insert was intact and that the organization of the functional elements within the insert, as present in plasmid pSYN12274, was maintained.

MATERIALS AND METHODS

Genetic Elements for 5307 maize in Plasmid pSYN12274

The genetic elements in plasmid pSYN12274, the 5307 maize transformation plasmid, are listed in Table 1 and mapped in Figure 1. The table also contains a description of each of the constituents of the plasmid pSYN12274, including the size in base pairs (bp) and the position within the plasmid.

Table 1. Genetic elements in plasmid pSYN12274

Active ingredient cassette			
Genetic element	**Size (bp)**	**Position**	**Description**
Intervening sequence	203	26 to 228	Intervening sequence with restriction sites used for cloning
CMP promoter	346	229 to 574	Cestrum Yellow Leaf Curling Virus promoter region (Hohn *et al*. 2007). Provides constitutive expression in maize.
Intervening sequence	9	575 to 583	Intervening sequence with restriction sites used for cloning
ecry3.1Ab	1962	584 to 2545	An engineered Cry gene active against certain corn rootworm (*Diabrotica*) species (Entrez® Accession No. GU327680 [NCBI 2010]). As an engineered chimeric protein, eCry3.1Ab has similarities to other well characterized Cry proteins. Because Cry proteins share structural similarities, chimeric Cry genes can be

Table 1. Genetic elements in plasmid pSYN12274 (*Continued*)

Genetic element	Size (bp)	Position	Description
			engineered *via* the exchange of domains that are homologous between different Cry genes. The gene *ecry3.1Ab* consists of a fusion between the 5′ end (Domain I, Domain II and 15 AA of Domain III) of a modified Cry3A gene (*mcry3A*) and the 3′ end (Domain III and Variable Region 6 [Hofte and Whiteley 1989]) of a synthetic Cry1Ab gene (see descriptions of *mcry3A* and *cry1Ab* below). Upstream of the *mcry3A* domain, the gene *ecry3.1Ab* carries a 67 bp long oligomer extension at its 5′ end, which was introduced during the engineering of the variable regions and is translated into the following 22 amino acid residues: MTSNGRQCAGIRPYDGRQQHRG. The next 459 amino acid residues are identical to those of mCry3A, followed by 172 residues of Cry1Ab. Description of *mcry3A*: a maize-optimized *cry3A* was synthesized to accommodate the preferred codon usage for maize (Murray *et al*. 1989). The synthetic sequence was based on the native Cry3A protein sequence from *Bacillus thuringiensis* subsp. *tenebrionis* (Sekar *et al*. 1987). The maize-optimized gene was then modified to incorporate a consensus cathepsin-G protease recognition site within the expressed protein. The amino acid sequence of the encoded mCry3A corresponds to that of the native Cry3A, except that (1) its N-terminus corresponds to methionine-48 of the native protein and (2) a cathepsin G protease recognition site has been introduced, beginning at amino acid residue 155 of the native protein. This cathepsin-G recognition site has the sequence alanine-alanine-proline-phenylalanine, and has replaced the amino acids valine-155, serine-156, and serine-157 in the native protein (Chen and Stacy 2007). Description of *cry1Ab*: the gene *cry1Ab* was originally cloned from *Bacillus thuringiensis* subsp. *kurstaki* strain HD-1 (Geiser *et al*. 1986). Its amino acid sequence has been codon-optimized (Koziel *et al*. 1997) to accommodate the preferred codon usage for maize (Murray *et al*. 1989)
Intervening sequence	30	2546 to 2575	Intervening sequence with restriction sites used for cloning

Table 1. Genetic elements in plasmid pSYN12274 (*Continued*)

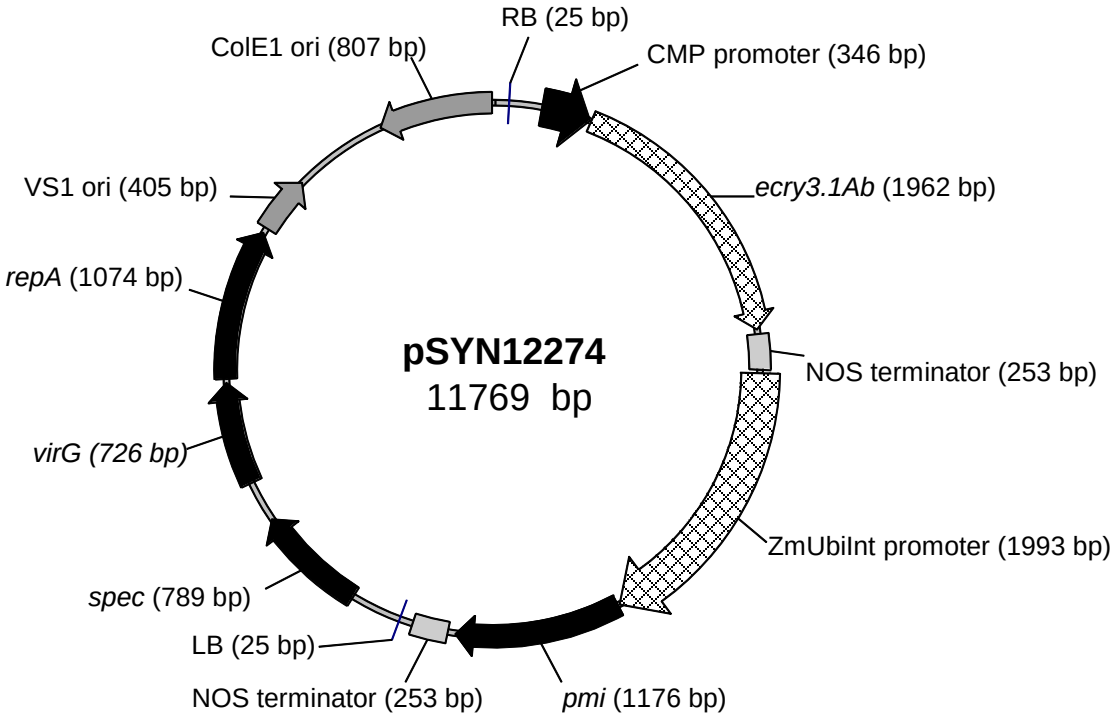
Genetic element	**Size (bp)**	**Position**	**Description**
NOS terminator	253	2576 to 2828	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens* (Entrez® Accession No. V00087 [NCBI 2010]). Provides a polyadenylation site (Depicker *et al*. 1982)
Selectable marker cassette			
Genetic element	**Size (bp)**	**Position**	**Description**
Intervening sequence	25	2829 to 2853	Intervening sequence with restriction sites used for cloning
ZmUbiInt promoter	1993	2854 to 4846	Promoter region from the maize polyubiquitin gene which contains the first intron (Entrez® Accession Number S94464 [NCBI 2010]). Provides constitutive expression in monocots (Christensen *et al*. 1992)
Intervening sequence	12	4847 to 4858	Intervening sequence with restriction sites used for cloning
pmi	1176	4859 to 6034	*Escherichia coli* gene *pmi* encoding the enzyme phosphomannose isomerase (PMI) (Entrez® Accession Number M15380 [NCBI 2010]); this gene is also known as *manA*. Catalyzes the isomerization of mannose-6-phosphate to fructose-6-phosphate (Negrotto *et al*. 2000)
Intervening sequence	60	6035 to 6094	Intervening sequence with restriction sites used for cloning
NOS terminator	253	6095 to 6347	Terminator sequence from the nopaline synthase gene of *Agrobacterium tumefaciens* (Entrez® Accession No. V00087 [NCBI 2010]). Provides a polyadenylation site (Depicker *et al*. 1982)
Intervening sequence	88	6348 to 6435	Intervening sequence with restriction sites used for cloning
Plasmid backbone			
Genetic element	**Size (bp)**	**Position**	**Description**
Left border (LB)	25	6436 to 6460	Left border region of T-DNA from *Agrobacterium tumefaciens* nopaline Ti-plasmid (Entrez® Accession Number J01825 [NCBI 2010]). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Zambryski *et al*. 1982)
Intervening sequence	349	6461 to 6809	Intervening sequence with restriction sites used for cloning

Table 1. Genetic elements in plasmid pSYN12274 (*Continued*)

Genetic element	Size (bp)	Position	Description
spec	789	6810 to 7598	Streptomycin adenylyltransferase, *aadA* gene from *Escherichia coli transposon* Tn7 (similar to Entrez® Accession Number X03043 [NCBI 2010]). Confers resistance to streptomycin and spectinomycin and is used as a bacterial selectable marker (Fling *et al*. 1985)
Intervening sequence	299	7599 to 7897	Intervening sequence with restriction sites used for cloning
virG	726	7898 to 8623	The VirGN54D gene (*virG*) from pAD1289 (similar to Entrez® Accession Number AF242881 [NCBI 2010]). The N54D substitution results in a constitutive *virG* phenotype. VirG is part of the two-component regulatory system for the virulence *(vir)* regulon in *Agrobacterium tumefaciens* (Hansen *et al*. 1994)
Intervening sequence	29	8624 to 8652	Intervening sequence with restriction sites used for cloning
repA	1074	8653 to 9726	Gene encoding the pVS1 replication protein from *Pseudomonas aeruginosa* (similar to Entrez® Accession Number AF133831 [NCBI 2010]), which is a part of the minimal pVS1 replicon that is functional in Gram-negative, plant-associated bacteria (Heeb *et al*. 2000)
Intervening sequence	42	9727 to 9768	Intervening sequence with restriction sites used for cloning
VS1 ori	405	9769 to 10173	Consensus sequence for the origin of replication and partitioning region from plasmid pVS1 of *Pseudomonas aeruginosa* (Entrez® Accession Number U10487 [NCBI 2010]). Serves as origin of replication in *Agrobacterium tumefaciens* host (Itoh *et al*. 1984)
Intervening sequence	677	10174 to 10850	Intervening sequence with restriction sites used for cloning
ColE1 ori	807	10851 to 11657	Origin of replication (similar to Entrez® Accession Number V00268 [NCBI 2010]) that permits replication of plasmids in *Escherichia coli* (Itoh and Tomizawa 1979)
Intervening sequence	112	11658 to 11769	Intervening sequence with restriction sites used for cloning
Right border (RB)	25	1 to 25	Right border region of T-DNA from *Agrobacterium tumefaciens* nopaline Ti-plasmid (Entrez® Accession Number J01826 [NCBI 2010]). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Wang *et al*. 1984)

T-DNA = transferred DNA

Figure 1. Plasmid map of plasmid pSYN12274



Test Substance

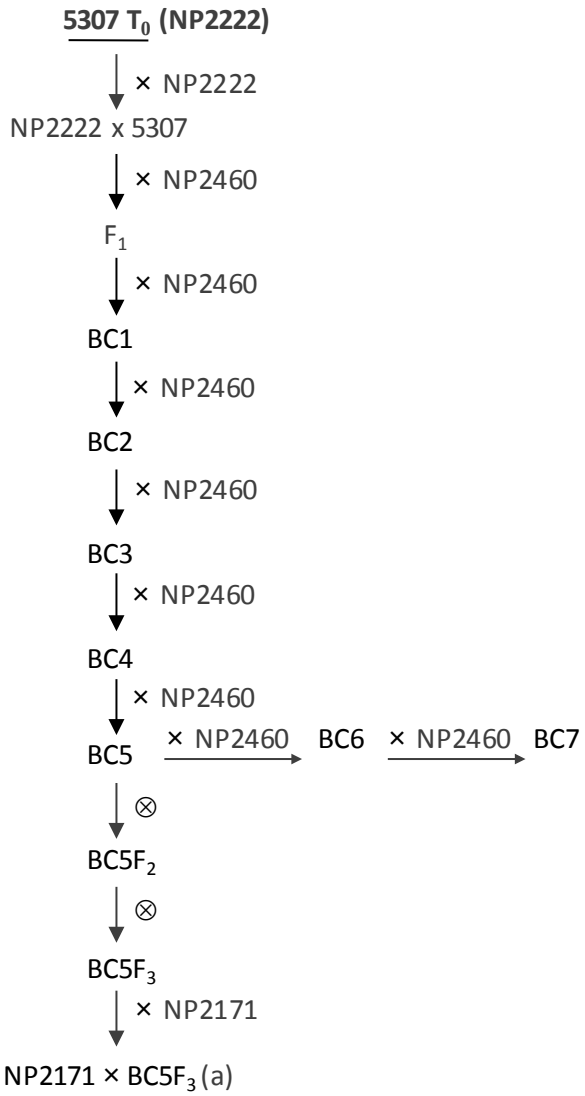
The test substance for this study was 5307 maize seed from generation NP2171 x $BC5F_3$. Table 2 shows the description and pedigree code for the test substance. Figure 2 illustrates a pedigree chart demonstrating the production of the test substance.

Table 2. Test substance

Seed identification	Pedigree
NP2171 x $BC5F_3$	NP2171 /(NP2460*//NP2222/(5307)1) B>B>B> B<2>B-B(T++)-

The test substance was characterized by real-time polymerase chain reaction (PCR) (Ingham *et al.* 2001) analysis to confirm the identity and purity.

Figure 2. Pedigree history for 5307 maize indicating the generation used in the study presented in this report



(a) = insert sequence analysis
T_0 = original transformant
× = cross
BC = backcross
⊗ = self-pollination

Plant Tissue for Genomic DNA Extraction

Test substance seed was grown in a Syngenta Biotechnology, Inc. greenhouse in Research Triangle Park, North Carolina, USA. Following verification of the plants' identity by real-time PCR analysis, leaf tissue from plants grown from the test substance was pooled into a sampling bag and stored at -80°C ± 10°C.

Genomic DNA Extraction

Genomic DNA used for insert sequence determination was isolated from the pooled leaf tissue using a modification of the method described in Saghai-Maroof *et al*. (1984).

Pooled leaf tissue was ground into a fine powder using a pre-chilled mortar and pestle, with liquid nitrogen, and then placed into a bottle for storage. For each DNA extraction, approximately 40 g of this tissue and 200 ml of prewarmed CTAB buffer (100 mM Tris pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 2% CTAB [w/v], 0.2% [v/v] β-mercaptoethanol) were combined in a bottle; the sample was then mixed gently and incubated for 90 minutes at 65°C ± 5°C. An equal volume of chloroform:isoamyl alcohol (24:1) was then added, followed by gentle mixing and centrifugation for 10 minutes at 7277 × *g* at room temperature.

The resulting aqueous phase was transferred to a clean container, and 10 µg of ribonuclease per ml of aqueous phase was added. The sample was mixed and incubated for 30 minutes at 37°C ± 2°C. An equal volume of chloroform:isoamyl alcohol (24:1) was then added, followed by gentle mixing and centrifugation for 10 minutes at 7277 × *g* at room temperature. The aqueous phase was collected in a clean bottle, and the DNA was precipitated with a 0.8 volume of isopropanol. The DNA was then pelleted by centrifugation at 291 × *g* and washed once with 70% ethanol. The DNA pellet was air-dried and dissolved in 2.5 ml of prewarmed 0.1X TE.

DNA Quantitation

The concentration of DNA was measured using a Quant-iT™ PicoGreen® dsDNA kit. A two-point standard curve was generated using a Lambda DNA standard. The linear attribute of the standard curve was verified with samples generated from a serial dilution of Lambda DNA standard in 1X TE. Genomic DNA was quantified by interpolation from the two point standard curve, and each genomic DNA was assayed in triplicate using the TBS-380 Mini-Fluorometer.

PCR Amplification

Two overlapping fragments that span the 5307 maize insert were amplified from genomic DNA extracted from 5307 maize using PCR analysis (Figure 3). PCR amplification was carried out using the Expand™ Long Template PCR System. Table 3 lists the primers used to amplify the insert fragments; Tables 4 and 5 contain the thermalcycling parameters.

Figure 3. Map of the 5307 maize insert and location of PCR-amplified fragments from 5307 maize to determine insert sequence

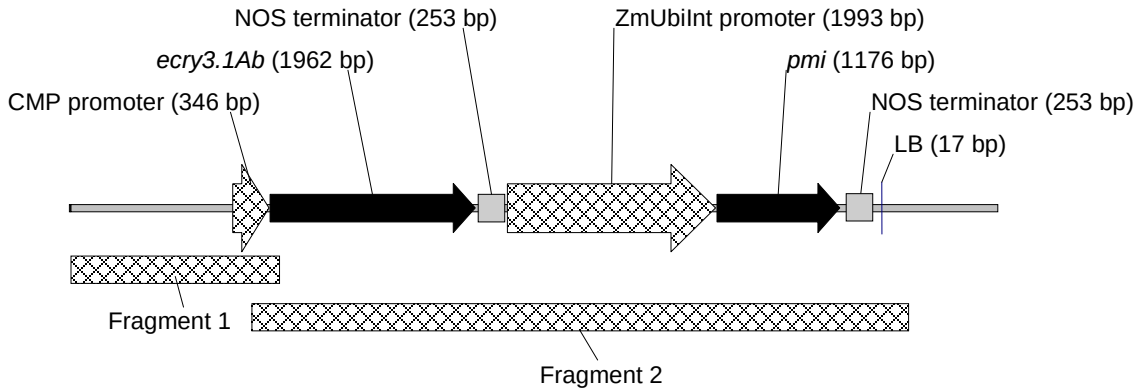


Table 3. Primers used to amplify the insert of 5307 maize

Fragment	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')
1	GTGTAAGCCCCAAGCCATTTACTTCCTC	CGTCCTTGGTGGTGCTGCTGTCCAGGC
2	ATTCGTGGCCGACAGGTGG	AGCCGTACTATAAAGAGGGGTTGTCG

Table 4. Cycling parameters for Fragment 1

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	94	2 min	1
B	1	94	15 sec	30
B	2	65	30 sec	30
B	3	72	90 sec	30
C	1	72	5 min	1
D	1	4	Hold	1

Table 5. Cycling parameters for Fragment 2

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	94	2 min	1
B	1	94	10 sec	1
B	2	60	30 sec	1
B	3	68	5 min	1
C	1	94	15 sec	25
C	2	60	30 sec	25
C	3	68	5 min (+20 sec each cycle)	25
D	1	68	7 min	1
E	1	4	Hold	1

The PCR fragments were cloned into pCR®4-TOPO® vector, and three colonies for each PCR product were randomly selected and grown. The plasmid DNA was then

independently extracted, and the resulting plasmid preparations, which contained the PCR amplification products, were subsequently sequenced.

Sequencing

Dye-terminator sequencing, a modification of the dideoxynucleotide chain-terminator sequencing method, was carried out using the ABI3730XL analyzer with ABI BigDye® 3.1 terminator chemistry. The sequence analysis was done using the Phred, Phrap, and Consed package (from the University of Washington), and was carried out to an error rate of less than 1 in 10,000 bases (Ewing and Green 1998).

Three individual clones for each PCR product were sequenced individually, and a consensus sequence was generated for each clone. These sequences were aligned using AlignX™, a component of Vector NTI Advance™, version 10.3.0, to obtain the final consensus sequence for each segment of the insert sequence.

Statistical Analysis

No statistical analysis was used during this study.

RESULTS AND DISCUSSION

Insert Sequencing

The consensus sequence data for the 5307 maize insert was compared to sequence of the transformation plasmid pSYN12274 (Figure 4). The data demonstrated that the insert was intact and that the organization of the functional elements within the insert, as present in plasmid pSYN12274, was maintained. One nucleotide change was determined in the 5307 maize insert 48 bp upstream of the CMP promoter in a non-coding region of the T-DNA (Figure 4). This nucleotide change has no effect on the genes encoded by 5307 maize.

The functional elements *ecry3.1Ab, pmi,* the CMP promoter, the ZmUbiInt promoter, and the NOS terminators in 5307 maize were identical to those in the transformation plasmid pSYN12274.

Sequence analysis revealed that some truncation occurred at the right border (RB) and left border (LB) ends of the transferred DNA (T-DNA) during the transformation process. The entire RB, three bp of non-coding sequence at the 5′ end of the insert, and eight bp of the LB were truncated. These deletions had no effect on the functionality of the insert as this phenomenon was previously observed in transformations with *Agrobacterium tumefaciens* (Tinland and Hohn 1995, Brunaud *et al.* 2002, Chilton and Que 2003).

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274

{CBI Cross Reference Number 1}

Data Quality and Integrity

No circumstances occurred during the conduct of this study that would have adversely affected the quality or integrity of the data generated.

CONCLUSIONS

Sequence analysis of the entire T-DNA present in 5307 maize confirmed that the insert remained intact and that the organization of the functional elements within the insert, as present in the transformation plasmid pSYN12274, was maintained. One nucleotide change was determined in the 5307 maize insert 48 bp upstream of the CMP promoter in a non-coding region of the T-DNA; however, this nucleotide change had no effect on the functionality of the T-DNA.

Sequence analysis revealed that some truncation occurred at the 5′ and 3′ ends of the T-DNA during the transformation process (resulting in 5307 maize). The entire RB, three bp of the non-coding sequence at the 5′ end of the insert, and eight bp of the LB were truncated; however, these deletions had no effect on the functionality of the T-DNA.

RECORDS RETENTION

Raw data, the original copy of this report, and other relevant records are archived at Syngenta Biotechnology, Inc., 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257, USA.

CONTRIBUTING SCIENTISTS

The analytical work reported herein was conducted by Stephen New, B.S., and Annick de Framond, PhD. This work was conducted at Syngenta Biotechnology, Inc.

Reported by: _____ November 3, 2010

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Event 5307 maize:

Insert Sequence Analysis

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Data Requirement(s):	Not applicable
Author:	Stephen New
Study Completion Date:	November 3, 2010
Performing Laboratory:	Syngenta Biotechnology, Inc. Product Safety 3054 East Cornwallis Road PO Box 12257 Research Triangle Park, NC 27709-2257, USA
Syngenta Study No.:	Not applicable
Report No.:	SSB-159-10 A1

CBI Cross-Reference Number 1	This cross-reference number noted on a place holder page is used in place of the following whole page at the indicated volume and page references.

Deleted pages are attached immediately behind this page.

Pages	Reason for Deletion	FIFRA Reference
18	Discloses information concerning the composition of the product	§10(d)(1)(A)

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274

The base pair discrepancy is indicated by the following text format: ▼ **G** **T**

```
                          RB
  pSYN12274     (1) GTTTACCCGCCAATATATCCTGTCAAACACTGATAGTTTAAACTGAAGGC
5307 insert     (1) ----------------------------ACTGATAGTTTAAACTGAAGGC


  pSYN12274    (51) GGGAAACGACAATCTGATCATGAGCGGAGAATTAAGGGAGTCACGTTATG
5307 insert    (23) GGGAAACGACAATCTGATCATGAGCGGAGAATTAAGGGAGTCACGTTATG


  pSYN12274   (101) ACCCCCGCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAACTGACAGA
5307 insert    (73) ACCCCCGCCGATGACGCGGGACAAGCCGTTTTACGTTTGGAACTGACAGA


                                                ▼
  pSYN12274   (151) ACCGCAACGCTGCAGGAATTGGCCGCAGCGGCCATTTAAATCAATTGGGC
5307 insert   (123) ACCGCAACGCTGCAGGAATTGGCCGCAGCTGCCATTTAAATCAATTGGGC


                                                CMP promoter
  pSYN12274   (201) GCGCCGAATTCGAGCTCGGTACAAGCTTCTGGCAGACAAAGTGGCAGACA
5307 insert   (173) GCGCCGAATTCGAGCTCGGTACAAGCTTCTGGCAGACAAAGTGGCAGACA


                                  CMP promoter
  pSYN12274   (251) TACTGTCCCACAAATGAAGATGGAATCTGTAAAAGAAAACGCGTGAAATA
5307 insert   (223) TACTGTCCCACAAATGAAGATGGAATCTGTAAAAGAAAACGCGTGAAATA


                                  CMP promoter
  pSYN12274   (301) ATGCGTCTGACAAAGGTTAGGTCGGCTGCCTTTAATCAATACCAAAGTGG
5307 insert   (273) ATGCGTCTGACAAAGGTTAGGTCGGCTGCCTTTAATCAATACCAAAGTGG


                                  CMP promoter
  pSYN12274   (351) TCCCTACCACGATGGAAAAACTGTGCAGTCGGTTTGGCTTTTTCTGACGA
5307 insert   (323) TCCCTACCACGATGGAAAAACTGTGCAGTCGGTTTGGCTTTTTCTGACGA


                                  CMP promoter
  pSYN12274   (401) ACAAATAAGATTCGTGGCCGACAGGTGGGGGTCCACCATGTGAAGGCATC
5307 insert   (373) ACAAATAAGATTCGTGGCCGACAGGTGGGGGTCCACCATGTGAAGGCATC


                                  CMP promoter
  pSYN12274   (451) TTCAGACTCCAATAATGGAGCAATGACGTAAGGGCTTACGAAATAAGTAA
5307 insert   (423) TTCAGACTCCAATAATGGAGCAATGACGTAAGGGCTTACGAAATAAGTAA


                                  CMP promoter
  pSYN12274   (501) GGGTAGTTTGGGAAATGTCCACTCACCCGTCAGTCTATAAATACTTAGCC
5307 insert   (473) GGGTAGTTTGGGAAATGTCCACTCACCCGTCAGTCTATAAATACTTAGCC


                         CMP promoter                    ecry3.1Ab
  pSYN12274   (551) CCTCCCTCATTGTTAAGGGAGCAAGGATCCACCATGACTAGTAACGGCCG
5307 insert   (523) CCTCCCTCATTGTTAAGGGAGCAAGGATCCACCATGACTAGTAACGGCCG
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

ecry3.1Ab

pSYN12274 (601) CCAGTGTGCTGGTATTCGCCCTTATGACGGCCGACAACAACACCGAGGCC
5307 insert (573) CCAGTGTGCTGGTATTCGCCCTTATGACGGCCGACAACAACACCGAGGCC

ecry3.1Ab

pSYN12274 (651) TGGACAGCAGCACCACCAAGGACGTGATCCAGAAGGGCATCAGCGTGGTG
5307 insert (623) TGGACAGCAGCACCACCAAGGACGTGATCCAGAAGGGCATCAGCGTGGTG

ecry3.1Ab

pSYN12274 (701) GGCGACCTGCTGGGCGTGGTGGGCTTCCCCTTCGGCGGCGCCCTGGTGAG
5307 insert (673) GGCGACCTGCTGGGCGTGGTGGGCTTCCCCTTCGGCGGCGCCCTGGTGAG

ecry3.1Ab

pSYN12274 (751) CTTCTACACCAACTTCCTGAACACCATCTGGCCCAGCGAGGACCCCTGGA
5307 insert (723) CTTCTACACCAACTTCCTGAACACCATCTGGCCCAGCGAGGACCCCTGGA

ecry3.1Ab

pSYN12274 (801) AGGCCTTCATGGAGCAGGTGGAGGCCCTGATGGACCAGAAGATCGCCGAC
5307 insert (773) AGGCCTTCATGGAGCAGGTGGAGGCCCTGATGGACCAGAAGATCGCCGAC

ecry3.1Ab

pSYN12274 (851) TACGCCAAGAACAAGGCACTGGCCGAGCTACAGGGCCTCCAGAACAACGT
5307 insert (823) TACGCCAAGAACAAGGCACTGGCCGAGCTACAGGGCCTCCAGAACAACGT

ecry3.1Ab

pSYN12274 (901) GGAGGACTATGTGAGCGCCCTGAGCAGCTGGCAGAAGAACCCCGCTGCAC
5307 insert (873) GGAGGACTATGTGAGCGCCCTGAGCAGCTGGCAGAAGAACCCCGCTGCAC

ecry3.1Ab

pSYN12274 (951) CGTTCCGCAACCCCCACAGCCAGGGCCGCATCCGCGAGCTGTTCAGCCAG
5307 insert (923) CGTTCCGCAACCCCCACAGCCAGGGCCGCATCCGCGAGCTGTTCAGCCAG

ecry3.1Ab

pSYN12274 (1001) GCCGAGAGCCACTTCCGCAACAGCATGCCCAGCTTCGCCATCAGCGGCTA
5307 insert (973) GCCGAGAGCCACTTCCGCAACAGCATGCCCAGCTTCGCCATCAGCGGCTA

ecry3.1Ab

pSYN12274 (1051) CGAGGTGCTGTTCCTGACCACCTACGCCCAGGCCGCCAACACCCACCTGT
5307 insert (1023) CGAGGTGCTGTTCCTGACCACCTACGCCCAGGCCGCCAACACCCACCTGT

ecry3.1Ab

pSYN12274 (1101) TCCTGCTGAAGGACGCCCAAATCTACGGAGAGGAGTGGGGCTACGAGAAG
5307 insert (1073) TCCTGCTGAAGGACGCCCAAATCTACGGAGAGGAGTGGGGCTACGAGAAG

ecry3.1Ab

pSYN12274 (1151) GAGGACATCGCCGAGTTCTACAAGCGCCAGCTGAAGCTGACCCAGGAGTA
5307 insert (1123) GAGGACATCGCCGAGTTCTACAAGCGCCAGCTGAAGCTGACCCAGGAGTA

ecry3.1Ab

pSYN12274 (1201) CACCGACCACTGCGTGAAGTGGTACAACGTGGGTCTAGACAAGCTCCGCG
5307 insert (1173) CACCGACCACTGCGTGAAGTGGTACAACGTGGGTCTAGACAAGCTCCGCG

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        ecry3.1Ab
  pSYN12274  (1251) GCAGCAGCTACGAGAGCTGGGTGAACTTCAACCGCTACCGCCGCGAGATG
5307 insert  (1223) GCAGCAGCTACGAGAGCTGGGTGAACTTCAACCGCTACCGCCGCGAGATG

                                        ecry3.1Ab
  pSYN12274  (1301) ACCCTGACCGTGCTGGACCTGATCGCCCTGTTCCCCCTGTACGACGTGCG
5307 insert  (1273) ACCCTGACCGTGCTGGACCTGATCGCCCTGTTCCCCCTGTACGACGTGCG

                                        ecry3.1Ab
  pSYN12274  (1351) CCTGTACCCCAAGGAGGTGAAGACCGAGCTGACCCGCGACGTGCTGACCG
5307 insert  (1323) CCTGTACCCCAAGGAGGTGAAGACCGAGCTGACCCGCGACGTGCTGACCG

                                        ecry3.1Ab
  pSYN12274  (1401) ACCCCATCGTGGGCGTGAACAACCTGCGCGGCTACGGCACCACCTTCAGC
5307 insert  (1373) ACCCCATCGTGGGCGTGAACAACCTGCGCGGCTACGGCACCACCTTCAGC

                                        ecry3.1Ab
  pSYN12274  (1451) AACATCGAGAACTACATCCGCAAGCCCCACCTGTTCGACTACCTGCACCG
5307 insert  (1423) AACATCGAGAACTACATCCGCAAGCCCCACCTGTTCGACTACCTGCACCG

                                        ecry3.1Ab
  pSYN12274  (1501) CATCCAGTTCCACACGCGTTTCCAGCCCGGCTACTACGGCAACGACAGCT
5307 insert  (1473) CATCCAGTTCCACACGCGTTTCCAGCCCGGCTACTACGGCAACGACAGCT

                                        ecry3.1Ab
  pSYN12274  (1551) TCAACTACTGGAGCGGCAACTACGTGAGCACCCGCCCCAGCATCGGCAGC
5307 insert  (1523) TCAACTACTGGAGCGGCAACTACGTGAGCACCCGCCCCAGCATCGGCAGC

                                        ecry3.1Ab
  pSYN12274  (1601) AACGACATCATCACCAGCCCCTTCTACGGCAACAAGAGCAGCGAGCCCGT
5307 insert  (1573) AACGACATCATCACCAGCCCCTTCTACGGCAACAAGAGCAGCGAGCCCGT

                                        ecry3.1Ab
  pSYN12274  (1651) GCAGAACCTTGAGTTCAACGGCGAGAAGGTGTACCGCGCCGTGGCTAACA
5307 insert  (1623) GCAGAACCTTGAGTTCAACGGCGAGAAGGTGTACCGCGCCGTGGCTAACA

                                        ecry3.1Ab
  pSYN12274  (1701) CCAACCTGGCCGTGTGGCCCTCTGCAGTGTACAGCGGCGTGACCAAGGTG
5307 insert  (1673) CCAACCTGGCCGTGTGGCCCTCTGCAGTGTACAGCGGCGTGACCAAGGTG

                                        ecry3.1Ab
  pSYN12274  (1751) GAGTTCAGCCAGTACAACGACCAGACCGACGAGGCCAGCACCCAGACCTA
5307 insert  (1723) GAGTTCAGCCAGTACAACGACCAGACCGACGAGGCCAGCACCCAGACCTA

                                        ecry3.1Ab
  pSYN12274  (1801) CGACAGCAAGCGCAACGTGGGCGCCGTGAGCTGGGACAGCATCGACCAGC
5307 insert  (1773) CGACAGCAAGCGCAACGTGGGCGCCGTGAGCTGGGACAGCATCGACCAGC

                                        ecry3.1Ab
  pSYN12274  (1851) TGCCCCCCGAGACCACCGACGAGCCCCTGGAGAAGGGCTACAGCCACCAG
5307 insert  (1823) TGCCCCCCGAGACCACCGACGAGCCCCTGGAGAAGGGCTACAGCCACCAG
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        ecry3.1Ab
  pSYN12274  (1901) CTGAACTACGTGATGTGCTTCCTGATGCAGGGCAGCCGCGGCACCATCCC
5307 insert  (1873) CTGAACTACGTGATGTGCTTCCTGATGCAGGGCAGCCGCGGCACCATCCC

                                        ecry3.1Ab
  pSYN12274  (1951) CGTGCTGACCTGGACCCACAAGAGCGTCGACTTCTTCAACATGATCGACA
5307 insert  (1923) CGTGCTGACCTGGACCCACAAGAGCGTCGACTTCTTCAACATGATCGACA

                                        ecry3.1Ab
  pSYN12274  (2001) GCAAGAAGATCACCCAGCTGCCCCTGACCAAGAGCACCAACCTGGGCAGC
5307 insert  (1973) GCAAGAAGATCACCCAGCTGCCCCTGACCAAGAGCACCAACCTGGGCAGC

                                        ecry3.1Ab
  pSYN12274  (2051) GGCACCAGCGTGGTGAAGGGCCCCGGCTTCACCGGCGGCGACATCCTGCG
5307 insert  (2023) GGCACCAGCGTGGTGAAGGGCCCCGGCTTCACCGGCGGCGACATCCTGCG

                                        ecry3.1Ab
  pSYN12274  (2101) CCGCACCAGCCCCGGCCAGATCAGCACCCTGCGCGTGAACATCACCGCCC
5307 insert  (2073) CCGCACCAGCCCCGGCCAGATCAGCACCCTGCGCGTGAACATCACCGCCC

                                        ecry3.1Ab
  pSYN12274  (2151) CCCTGAGCCAGCGCTACCGCGTCCGCATCCGCTACGCCAGCACCACCAAC
5307 insert  (2123) CCCTGAGCCAGCGCTACCGCGTCCGCATCCGCTACGCCAGCACCACCAAC

                                        ecry3.1Ab
  pSYN12274  (2201) CTGCAGTTCCACACCAGCATCGACGGCCGCCCCATCAACCAGGGCAACTT
5307 insert  (2173) CTGCAGTTCCACACCAGCATCGACGGCCGCCCCATCAACCAGGGCAACTT

                                        ecry3.1Ab
  pSYN12274  (2251) CAGCGCCACCATGAGCAGCGGCAGCAACCTGCAGAGCGGCAGCTTCCGCA
5307 insert  (2223) CAGCGCCACCATGAGCAGCGGCAGCAACCTGCAGAGCGGCAGCTTCCGCA

                                        ecry3.1Ab
  pSYN12274  (2301) CCGTGGGCTTCACCACCCCCTTCAACTTCAGCAACGGCAGCAGCGTGTTC
5307 insert  (2273) CCGTGGGCTTCACCACCCCCTTCAACTTCAGCAACGGCAGCAGCGTGTTC

                                        ecry3.1Ab
  pSYN12274  (2351) ACCCTGAGCGCCCACGTGTTCAACAGCGGCAACGAGGTGTACATCGACCG
5307 insert  (2323) ACCCTGAGCGCCCACGTGTTCAACAGCGGCAACGAGGTGTACATCGACCG

                                        ecry3.1Ab
  pSYN12274  (2401) CATCGAGTTCGTGCCCGCCGAGGTGACCTTCGAGGCCGAGTACGACCTGG
5307 insert  (2373) CATCGAGTTCGTGCCCGCCGAGGTGACCTTCGAGGCCGAGTACGACCTGG

                                        ecry3.1Ab
  pSYN12274  (2451) AGAGGGCTCAGAAGGCCGTGAACGAGCTGTTCACCAGCAGCAACCAGATC
5307 insert  (2423) AGAGGGCTCAGAAGGCCGTGAACGAGCTGTTCACCAGCAGCAACCAGATC

                                      ecry3.1Ab
  pSYN12274  (2501) GGCCTGAAGACCGACGTGACCGACTACCACATCGATCAGGTGTAGGAGCT
5307 insert  (2473) GGCCTGAAGACCGACGTGACCGACTACCACATCGATCAGGTGTAGGAGCT
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                                                NOS terminator
  pSYN12274  (2551) GAGCTCTAGATCCCCGAATTTCCCCGATCGTTCAAACATTTGGCAATAAA
5307 insert  (2523) GAGCTCTAGATCCCCGAATTTCCCCGATCGTTCAAACATTTGGCAATAAA

                                  NOS terminator
  pSYN12274  (2601) GTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAA
5307 insert  (2573) GTTTCTTAAGATTGAATCCTGTTGCCGGTCTTGCGATGATTATCATATAA

                                  NOS terminator
  pSYN12274  (2651) TTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGAC
5307 insert  (2623) TTTCTGTTGAATTACGTTAAGCATGTAATAATTAACATGTAATGCATGAC

                                  NOS terminator
  pSYN12274  (2701) GTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTT
5307 insert  (2673) GTTATTTATGAGATGGGTTTTTATGATTAGAGTCCCGCAATTATACATTT

                                  NOS terminator
  pSYN12274  (2751) AATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCG
5307 insert  (2723) AATACGCGATAGAAAACAAAATATAGCGCGCAAACTAGGATAAATTATCG

                          NOS_terminator
  pSYN12274  (2801) CGCGCGGTGTCATCTATGTTACTAGATCGGGAATTGGGTACCAGCTTGCA
5307 insert  (2773) CGCGCGGTGTCATCTATGTTACTAGATCGGGAATTGGGTACCAGCTTGCA

                                  ZmUbiInt promoter
  pSYN12274  (2851) TGCCTGCAGTGCAGCGTGACCCGGTCGTGCCCCTCTCTAGAGATAATGAG
5307 insert  (2823) TGCCTGCAGTGCAGCGTGACCCGGTCGTGCCCCTCTCTAGAGATAATGAG

                                  ZmUbiInt promoter
  pSYN12274  (2901) CATTGCATGTCTAAGTTATAAAAAATTACCACATATTTTTTTTGTCACAC
5307 insert  (2873) CATTGCATGTCTAAGTTATAAAAAATTACCACATATTTTTTTTGTCACAC

                                  ZmUbiInt promoter
  pSYN12274  (2951) TTGTTTGAAGTGCAGTTTATCTATCTTTATACATATATTTAAACTTTACT
5307 insert  (2923) TTGTTTGAAGTGCAGTTTATCTATCTTTATACATATATTTAAACTTTACT

                                  ZmUbiInt promoter
  pSYN12274  (3001) CTACGAATAATATAATCTATAGTACTACAATAATATCAGTGTTTTAGAGA
5307 insert  (2973) CTACGAATAATATAATCTATAGTACTACAATAATATCAGTGTTTTAGAGA

                                  ZmUbiInt promoter
  pSYN12274  (3051) ATCATATAAATGAACAGTTAGACATGGTCTAAAGGACAATTGAGTATTTT
5307 insert  (3023) ATCATATAAATGAACAGTTAGACATGGTCTAAAGGACAATTGAGTATTTT

                                  ZmUbiInt promoter
  pSYN12274  (3101) GACAACAGGACTCTACAGTTTTATCTTTTTAGTGTGCATGTGTTCTCCTT
5307 insert  (3073) GACAACAGGACTCTACAGTTTTATCTTTTTAGTGTGCATGTGTTCTCCTT

                                  ZmUbiInt promoter
  pSYN12274  (3151) TTTTTTTGCAAATAGCTTCACCTATATAATACTTCATCCATTTTATTAGT
5307 insert  (3123) TTTTTTTGCAAATAGCTTCACCTATATAATACTTCATCCATTTTATTAGT
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                    ZmUbiInt promoter
  pSYN12274  (3201) ACATCCATTTAGGGTTTAGGGTTAATGGTTTTTATAGACTAATTTTTTTA
5307 insert  (3173) ACATCCATTTAGGGTTTAGGGTTAATGGTTTTTATAGACTAATTTTTTTA

                                    ZmUbiInt promoter
  pSYN12274  (3251) GTACATCTATTTTATTCTATTTTAGCCTCTAAATTAAGAAAACTAAAACT
5307 insert  (3223) GTACATCTATTTTATTCTATTTTAGCCTCTAAATTAAGAAAACTAAAACT

                                    ZmUbiInt promoter
  pSYN12274  (3301) CTATTTTAGTTTTTTTATTTAATAATTTAGATATAAAATAGAATAAAATA
5307 insert  (3273) CTATTTTAGTTTTTTTATTTAATAATTTAGATATAAAATAGAATAAAATA

                                    ZmUbiInt promoter
  pSYN12274  (3351) AAGTGACTAAAAATTAAACAAATACCCTTTAAGAAATTAAAAAAACTAAG
5307 insert  (3323) AAGTGACTAAAAATTAAACAAATACCCTTTAAGAAATTAAAAAAACTAAG

                                    ZmUbiInt promoter
  pSYN12274  (3401) GAAACATTTTTCTTGTTTCGAGTAGATAATGCCAGCCTGTTAAACGCCGT
5307 insert  (3373) GAAACATTTTTCTTGTTTCGAGTAGATAATGCCAGCCTGTTAAACGCCGT

                                    ZmUbiInt promoter
  pSYN12274  (3451) CGACGAGTCTAACGGACACCAACCAGCGAACCAGCAGCGTCGCGTCGGGC
5307 insert  (3423) CGACGAGTCTAACGGACACCAACCAGCGAACCAGCAGCGTCGCGTCGGGC

                                    ZmUbiInt promoter
  pSYN12274  (3501) CAAGCGAAGCAGACGGCACGGCATCTCTGTCGCTGCCTCTGGACCCCTCT
5307 insert  (3473) CAAGCGAAGCAGACGGCACGGCATCTCTGTCGCTGCCTCTGGACCCCTCT

                                    ZmUbiInt promoter
  pSYN12274  (3551) CGAGAGTTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATCCAGAAA
5307 insert  (3523) CGAGAGTTCCGCTCCACCGTTGGACTTGCTCCGCTGTCGGCATCCAGAAA

                                    ZmUbiInt promoter
  pSYN12274  (3601) TTGCGTGGCGGAGCGGCAGACGTGAGCCGGCACGGCAGGCGGCCTCCTCC
5307 insert  (3573) TTGCGTGGCGGAGCGGCAGACGTGAGCCGGCACGGCAGGCGGCCTCCTCC

                                    ZmUbiInt promoter
  pSYN12274  (3651) TCCTCTCACGGCACCGGCAGCTACGGGGGATTCCTTTCCCACCGCTCCTT
5307 insert  (3623) TCCTCTCACGGCACCGGCAGCTACGGGGGATTCCTTTCCCACCGCTCCTT

                                    ZmUbiInt promoter
  pSYN12274  (3701) CGCTTTCCCTTCCTCGCCCGCCGTAATAAATAGACACCCCCTCCACACCC
5307 insert  (3673) CGCTTTCCCTTCCTCGCCCGCCGTAATAAATAGACACCCCCTCCACACCC

                                    ZmUbiInt promoter
  pSYN12274  (3751) TCTTTCCCCAACCTCGTGTTGTTCGGAGCGCACACACACACAACCAGATC
5307 insert  (3723) TCTTTCCCCAACCTCGTGTTGTTCGGAGCGCACACACACACAACCAGATC

                                    ZmUbiInt promoter
  pSYN12274  (3801) TCCCCCAAATCCACCCGTCGGCACCTCCGCTTCAAGGTACGCCGCTCGTC
5307 insert  (3773) TCCCCCAAATCCACCCGTCGGCACCTCCGCTTCAAGGTACGCCGCTCGTC
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                    ZmUbiInt promoter
  pSYN12274  (3851) CTCCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGTTCCGGTCCATG
5307 insert  (3823) CTCCCCCCCCCCCCCTCTCTACCTTCTCTAGATCGGCGTTCCGGTCCATG

                                    ZmUbiInt promoter
  pSYN12274  (3901) GTTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGATCCGTGT
5307 insert  (3873) GTTAGGGCCCGGTAGTTCTACTTCTGTTCATGTTTGTGTTAGATCCGTGT

                                    ZmUbiInt promoter
  pSYN12274  (3951) TTGTGTTAGATCCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACG
5307 insert  (3923) TTGTGTTAGATCCGTGCTGCTAGCGTTCGTACACGGATGCGACCTGTACG

                                    ZmUbiInt promoter
  pSYN12274  (4001) TCAGACACGTTCTGATTGCTAACTTGCCAGTGTTTCTCTTTGGGGAATCC
5307 insert  (3973) TCAGACACGTTCTGATTGCTAACTTGCCAGTGTTTCTCTTTGGGGAATCC

                                    ZmUbiInt promoter
  pSYN12274  (4051) TGGGATGGCTCTAGCCGTTCCGCAGACGGGATCGATTTCATGATTTTTTT
5307 insert  (4023) TGGGATGGCTCTAGCCGTTCCGCAGACGGGATCGATTTCATGATTTTTTT

                                    ZmUbiInt promoter
  pSYN12274  (4101) TGTTTCGTTGCATAGGGTTTGGTTTGCCCTTTTCCTTTATTTCAATATAT
5307 insert  (4073) TGTTTCGTTGCATAGGGTTTGGTTTGCCCTTTTCCTTTATTTCAATATAT

                                    ZmUbiInt promoter
  pSYN12274  (4151) GCCGTGCACTTGTTTGTCGGGTCATCTTTTCATGCTTTTTTTTGTCTTGG
5307 insert  (4123) GCCGTGCACTTGTTTGTCGGGTCATCTTTTCATGCTTTTTTTTGTCTTGG

                                    ZmUbiInt promoter
  pSYN12274  (4201) TTGTGATGATGTGGTCTGGTTGGGCGGTCGTTCTAGATCGGAGTAGAATT
5307 insert  (4173) TTGTGATGATGTGGTCTGGTTGGGCGGTCGTTCTAGATCGGAGTAGAATT

                                    ZmUbiInt promoter
  pSYN12274  (4251) CTGTTTCAAACTACCTGGTGGATTTATTAATTTTGGATCTGTATGTGTGT
5307 insert  (4223) CTGTTTCAAACTACCTGGTGGATTTATTAATTTTGGATCTGTATGTGTGT

                                    ZmUbiInt promoter
  pSYN12274  (4301) GCCATACATATTCATAGTTACGAATTGAAGATGATGGATGGAAATATCGA
5307 insert  (4273) GCCATACATATTCATAGTTACGAATTGAAGATGATGGATGGAAATATCGA

                                    ZmUbiInt promoter
  pSYN12274  (4351) TCTAGGATAGGTATACATGTTGATGCGGGTTTTACTGATGCATATACAGA
5307 insert  (4323) TCTAGGATAGGTATACATGTTGATGCGGGTTTTACTGATGCATATACAGA

                                    ZmUbiInt promoter
  pSYN12274  (4401) GATGCTTTTTGTTCGCTTGGTTGTGATGATGTGGTGTGGTTGGGCGGTCG
5307 insert  (4373) GATGCTTTTTGTTCGCTTGGTTGTGATGATGTGGTGTGGTTGGGCGGTCG

                                    ZmUbiInt promoter
  pSYN12274  (4451) TTCATTCGTTCTAGATCGGAGTAGAATACTGTTTCAAACTACCTGGTGTA
5307 insert  (4423) TTCATTCGTTCTAGATCGGAGTAGAATACTGTTTCAAACTACCTGGTGTA
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                    ZmUbiInt promoter
  pSYN12274  (4501) TTTATTAATTTTGGAACTGTATGTGTGTCATACATCTTCATAGTTACG
5307 insert  (4473) TTTATTAATTTTGGAACTGTATGTGTGTCATACATCTTCATAGTTACG

                                    ZmUbiInt promoter
  pSYN12274  (4551) AGTTTAAGATGGATGGAAATATCGATCTAGGATAGGTATACATGTTGATG
5307 insert  (4523) AGTTTAAGATGGATGGAAATATCGATCTAGGATAGGTATACATGTTGATG

                                    ZmUbiInt promoter
  pSYN12274  (4601) TGGGTTTTACTGATGCATATACATGATGGCATATGCAGCATCTATTCATA
5307 insert  (4573) TGGGTTTTACTGATGCATATACATGATGGCATATGCAGCATCTATTCATA

                                    ZmUbiInt promoter
  pSYN12274  (4651) TGCTCTAACCTTGAGTACCTATCTATTATAATAAACAAGTATGTTTTATA
5307 insert  (4623) TGCTCTAACCTTGAGTACCTATCTATTATAATAAACAAGTATGTTTTATA

                                    ZmUbiInt promoter
  pSYN12274  (4701) ATTATTTTGATCTTGATATACTTGGATGATGGCATATGCAGCAGCTATAT
5307 insert  (4673) ATTATTTTGATCTTGATATACTTGGATGATGGCATATGCAGCAGCTATAT

                                    ZmUbiInt promoter
  pSYN12274  (4751) GTGGATTTTTTTAGCCCTGCCTTCATACGCTATTTATTTGCTTGGTACTG
5307 insert  (4723) GTGGATTTTTTTAGCCCTGCCTTCATACGCTATTTATTTGCTTGGTACTG

                                  ZmUbiInt promoter
  pSYN12274  (4801) TTTCTTTTGTCGATGCTCACCCTGTTGTTTGGTGTTACTTCTGCAGGGAT
5307 insert  (4773) TTTCTTTTGTCGATGCTCACCCTGTTGTTTGGTGTTACTTCTGCAGGGAT

                                        pmi
  pSYN12274  (4851) CCCCGATCATGCAAAAACTCATTAACTCAGTGCAAAACTATGCCTGGGGC
5307 insert  (4823) CCCCGATCATGCAAAAACTCATTAACTCAGTGCAAAACTATGCCTGGGGC

                                     pmi
  pSYN12274  (4901) AGCAAAACGGCGTTGACTGAACTTTATGGTATGGAAAATCCGTCCAGCCA
5307 insert  (4873) AGCAAAACGGCGTTGACTGAACTTTATGGTATGGAAAATCCGTCCAGCCA

                                     pmi
  pSYN12274  (4951) GCCGATGGCCGAGCTGTGGATGGGCGCACATCCGAAAAGCAGTTCACGAG
5307 insert  (4923) GCCGATGGCCGAGCTGTGGATGGGCGCACATCCGAAAAGCAGTTCACGAG

                                     pmi
  pSYN12274  (5001) TGCAGAATGCCGCCGGAGATATCGTTTCACTGCGTGATGTGATTGAGAGT
5307 insert  (4973) TGCAGAATGCCGCCGGAGATATCGTTTCACTGCGTGATGTGATTGAGAGT

                                     pmi
  pSYN12274  (5051) GATAAATCGACTCTGCTCGGAGAGGCCGTTGCCAAACGCTTTGGCGAACT
5307 insert  (5023) GATAAATCGACTCTGCTCGGAGAGGCCGTTGCCAAACGCTTTGGCGAACT

                                     pmi
  pSYN12274  (5101) GCCTTTCCTGTTCAAAGTATTATGCGCAGCACAGCCACTCTCCATTCAGG
5307 insert  (5073) GCCTTTCCTGTTCAAAGTATTATGCGCAGCACAGCCACTCTCCATTCAGG
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        pmi
  pSYN12274  (5151) TTCATCCAAACAAACACAATTCTGAAATCGGTTTTGCCAAAGAAAATGCC
5307 insert  (5123) TTCATCCAAACAAACACAATTCTGAAATCGGTTTTGCCAAAGAAAATGCC

                                        pmi
  pSYN12274  (5201) GCAGGTATCCCGATGGATGCCGCCGAGCGTAACTATAAAGATCCTAACCA
5307 insert  (5173) GCAGGTATCCCGATGGATGCCGCCGAGCGTAACTATAAAGATCCTAACCA

                                        pmi
  pSYN12274  (5251) CAAGCCGGAGCTGGTTTTTGCGCTGACGCCTTTCCTTGCGATGAACGCGT
5307 insert  (5223) CAAGCCGGAGCTGGTTTTTGCGCTGACGCCTTTCCTTGCGATGAACGCGT

                                        pmi
  pSYN12274  (5301) TTCGTGAATTTTCCGAGATTGTCTCCCTACTCCAGCCGGTCGCAGGTGCA
5307 insert  (5273) TTCGTGAATTTTCCGAGATTGTCTCCCTACTCCAGCCGGTCGCAGGTGCA

                                        pmi
  pSYN12274  (5351) CATCCGGCGATTGCTCACTTTTTACAACAGCCTGATGCCGAACGTTTAAG
5307 insert  (5323) CATCCGGCGATTGCTCACTTTTTACAACAGCCTGATGCCGAACGTTTAAG

                                        pmi
  pSYN12274  (5401) CGAACTGTTCGCCAGCCTGTTGAATATGCAGGGTGAAGAAAAATCCCGCG
5307 insert  (5373) CGAACTGTTCGCCAGCCTGTTGAATATGCAGGGTGAAGAAAAATCCCGCG

                                        pmi
  pSYN12274  (5451) CGCTGGCGATTTTAAAATCGGCCCTCGATAGCCAGCAGGGTGAACCGTGG
5307 insert  (5423) CGCTGGCGATTTTAAAATCGGCCCTCGATAGCCAGCAGGGTGAACCGTGG

                                        pmi
  pSYN12274  (5501) CAAACGATTCGTTTAATTTCTGAATTTTACCCGGAAGACAGCGGTCTGTT
5307 insert  (5473) CAAACGATTCGTTTAATTTCTGAATTTTACCCGGAAGACAGCGGTCTGTT

                                        pmi
  pSYN12274  (5551) CTCCCCGCTATTGCTGAATGTGGTGAAATTGAACCCTGGCGAAGCGATGT
5307 insert  (5523) CTCCCCGCTATTGCTGAATGTGGTGAAATTGAACCCTGGCGAAGCGATGT

                                        pmi
  pSYN12274  (5601) TCCTGTTCGCTGAAACACCGCACGCTTACCTGCAAGGCGTGGCGCTGGAA
5307 insert  (5573) TCCTGTTCGCTGAAACACCGCACGCTTACCTGCAAGGCGTGGCGCTGGAA

                                        pmi
  pSYN12274  (5651) GTGATGGCAAACTCCGATAACGTGCTGCGTGCGGGTCTGACGCCTAAATA
5307 insert  (5623) GTGATGGCAAACTCCGATAACGTGCTGCGTGCGGGTCTGACGCCTAAATA

                                        pmi
  pSYN12274  (5701) CATTGATATTCCGGAACTGGTTGCCAATGTGAAATTCGAAGCCAAACCGG
5307 insert  (5673) CATTGATATTCCGGAACTGGTTGCCAATGTGAAATTCGAAGCCAAACCGG

                                        pmi
  pSYN12274  (5751) CTAACCAGTTGTTGACCCAGCCGGTGAAACAAGGTGCAGAACTGGACTTC
5307 insert  (5723) CTAACCAGTTGTTGACCCAGCCGGTGAAACAAGGTGCAGAACTGGACTTC
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        pmi
  pSYN12274  (5801) CCGATTCCAGTGGATGATTTTGCCTTCTCGCTGCATGACCTTAGTGATAA
5307 insert  (5773) CCGATTCCAGTGGATGATTTTGCCTTCTCGCTGCATGACCTTAGTGATAA

                                        pmi
  pSYN12274  (5851) AGAAACCACCATTAGCCAGCAGAGTGCCGCCATTTTGTTCTGCGTCGAAG
5307 insert  (5823) AGAAACCACCATTAGCCAGCAGAGTGCCGCCATTTTGTTCTGCGTCGAAG

                                        pmi
  pSYN12274  (5901) GCGATGCAACGTTGTGGAAAGGTTCTCAGCAGTTACAGCTTAAACCGGGT
5307 insert  (5873) GCGATGCAACGTTGTGGAAAGGTTCTCAGCAGTTACAGCTTAAACCGGGT

                                        pmi
  pSYN12274  (5951) GAATCAGCGTTTATTGCCGCCAACGAATCACCGGTGACTGTCAAAGGCCA
5307 insert  (5923) GAATCAGCGTTTATTGCCGCCAACGAATCACCGGTGACTGTCAAAGGCCA

                                    pmi
  pSYN12274  (6001) CGGCCGTTTAGCGCGTGTTTACAACAAGCTGTAAGAGCTTACTGAAAAAA
5307 insert  (5973) CGGCCGTTTAGCGCGTGTTTACAACAAGCTGTAAGAGCTTACTGAAAAAA

                                                          NOS terminator
  pSYN12274  (6051) TTAACATCTCTTGCTAAGCTGGGAGCTCGATCCGTCGACCTGCAGATCGT
5307 insert  (6023) TTAACATCTCTTGCTAAGCTGGGAGCTCGATCCGTCGACCTGCAGATCGT

                                    NOS terminator
  pSYN12274  (6101) TCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCT
5307 insert  (6073) TCAAACATTTGGCAATAAAGTTTCTTAAGATTGAATCCTGTTGCCGGTCT

                                    NOS terminator
  pSYN12274  (6151) TGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAA
5307 insert  (6123) TGCGATGATTATCATATAATTTCTGTTGAATTACGTTAAGCATGTAATAA

                                    NOS terminator
  pSYN12274  (6201) TTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGA
5307 insert  (6173) TTAACATGTAATGCATGACGTTATTTATGAGATGGGTTTTTATGATTAGA

                                    NOS terminator
  pSYN12274  (6251) GTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGC
5307 insert  (6223) GTCCCGCAATTATACATTTAATACGCGATAGAAAACAAAATATAGCGCGC

                                  NOS_terminator
  pSYN12274  (6301) AAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTGC
5307 insert  (6273) AAACTAGGATAAATTATCGCGCGCGGTGTCATCTATGTTACTAGATCTGC


  pSYN12274  (6351) TAGCCCTGCAGGAAATTTACCGGTGCCCGGGCGGCCAGCATGGCCGTATC
5307 insert  (6323) TAGCCCTGCAGGAAATTTACCGGTGCCCGGGCGGCCAGCATGGCCGTATC

                                                                LB
  pSYN12274  (6401) CGCAATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATA
5307 insert  (6373) CGCAATGTGTTATTAAGTTGTCTAAGCGTCAATTTGTTTACACCACAATA
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                LB
  pSYN12274  (6451) TATCCTGCCACCAGCCAGCCAACAGCTCCCCGACCGGCAGCTCGGCACAA
5307 insert  (6423) TA-----------------------------------------------

  pSYN12274  (6501) AATCACCACTCGATACAGGCAGCCCATCAGAATTAATTCTCATGTTTGAC
5307 insert         -----------------------------------------------

  pSYN12274  (6551) AGCTTATCATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCC
5307 insert         -----------------------------------------------

  pSYN12274  (6601) ATCGGAAGCTGTGGTATGGCTGTGCAGGTCGTAAATCACTGCATAATTCG
5307 insert         -----------------------------------------------

  pSYN12274  (6651) TGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACAT
5307 insert         -----------------------------------------------

  pSYN12274  (6701) CATAACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCAT
5307 insert         -----------------------------------------------

  pSYN12274  (6751) CCGGCTCGTATAATGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG
5307 insert         -----------------------------------------------

                                          spec
  pSYN12274  (6801) AAACAGACCATGAGGGAAGCGTTGATCGCCGAAGTATCGACTCAACTATC
5307 insert         -----------------------------------------------

                                    spec
  pSYN12274  (6851) AGAGGTAGTTGGCGTCATCGAGCGCCATCTCGAACCGACGTTGCTGGCCG
5307 insert         -----------------------------------------------

                                    spec
  pSYN12274  (6901) TACATTTGTACGGCTCCGCAGTGGATGGCGGCCTGAAGCCACACAGTGAT
5307 insert         -----------------------------------------------

                                    spec
  pSYN12274  (6951) ATTGATTTGCTGGTTACGGTGACCGTAAGGCTTGATGAAACAACGCGGCG
5307 insert         -----------------------------------------------

                                    spec
  pSYN12274  (7001) AGCTTTGATCAACGACCTTTTGGAAACTTCGGCTTCCCCTGGAGAGAGCG
5307 insert         -----------------------------------------------

                                    spec
  pSYN12274  (7051) AGATTCTCCGCGCTGTAGAAGTCACCATTGTTGTGCACGACGACATCATT
5307 insert         -----------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        spec
  pSYN12274  (7101) CCGTGGCGTTATCCAGCTAAGCGCGAACTGCAATTTGGAGAATGGCAGCG
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7151) CAATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATC
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7201) TGGCTATCTTGCTGACAAAAGCAAGAGAACATAGCGTTGCCTTGGTAGGT
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7251) CCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGATCTATTTGA
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7301) GGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTG
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7351) GCGATGAGCGAAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCA
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7401) GTAACCGGCAAAATCGCGCCGAAGGATGTCGCTGCCGACTGGGCAATGGA
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7451) GCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGGCAGGCTT
5307 insert         --------------------------------------------------

                                        spec
  pSYN12274  (7501) ATCTTGGACAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAA
5307 insert         --------------------------------------------------

                                       spec
  pSYN12274  (7551) TTTGTTCACTACGTGAAAGGCGAGATCACCAAAGTAGTCGGCAAATAAAG
5307 insert         --------------------------------------------------

  pSYN12274  (7601) CTCTAGTGGATCTCCGTACCCGGGGATCTGGCTCGCGGCGGACGCACGAC
5307 insert         --------------------------------------------------

  pSYN12274  (7651) GCCGGGGCGAGACCATAGGCGATCTCCTAAATCAATAGTAGCTGTAACCT
5307 insert         --------------------------------------------------

  pSYN12274  (7701) CGAAGCGTTTCACTTGTAACAACGATTGAGAATTTTTGTCATAAAATTGA
5307 insert         --------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
  pSYN12274  (7751) AATACTTGGTTCGCATTTTTGTCATCCGCGGTCAGCCGCAATTCTGACGA
5307 insert         ------------------------------------------------


  pSYN12274  (7801) ACTGCCCATTTAGCTGGAGATGATTGTACATCCTTCACGTGAAAATTTCT
5307 insert         ------------------------------------------------

                                                                virG
  pSYN12274  (7851) CAAGCGCTGTGAACAAGGGTTCAGATTTTAGATTGAAAGGTGAGCCGTTG
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (7901) AAACACGTTCTTCTTGTCGATGACGACGTCGCTATGCGGCATCTTATTAT
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (7951) TGAATACCTTACGATCCACGCCTTCAAAGTGACCGCGGTAGCCGACAGCA
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8001) CCCAGTTCACAAGAGTACTCTCTTCCGCGACGGTCGATGTCGTGGTTGTT
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8051) GATCTAGATTTAGGTCGTGAAGATGGGCTCGAGATCGTTCGTAATCTGGC
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8101) GGCAAAGTCTGATATTCCAATCATAATTATCAGTGGCGACCGCCTTGAGG
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8151) AGACGGATAAAGTTGTTGCACTCGAGCTAGGAGCAAGTGATTTTATCGCT
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8201) AAGCCGTTCAGTATCAGAGAGTTTCTAGCACGCATTCGGGTTGCCTTGCG
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8251) CGTGCGCCCCAACGTTGTCCGCTCCAAAGACCGACGGTCTTTTTGTTTTA
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8301) CTGACTGGACACTTAATCTCAGGCAACGTCGCTTGATGTCCGAAGCTGGC
5307 insert         ------------------------------------------------

                                  virG
  pSYN12274  (8351) GGTGAGGTGAAACTTACGGCAGGTGAGTTCAATCTTCTCCTCGCGTTTTT
5307 insert         ------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        virG
  pSYN12274  (8401) AGAGAAACCCCGCGACGTTCTATCGCGCGAGCAACTTCTCATTGCCAGTC
5307 insert         ---------------------------------------------------

                                        virG
  pSYN12274  (8451) GAGTACGCGACGAGGAGGTTTATGACAGGAGTATAGATGTTCTCATTTTG
5307 insert         ---------------------------------------------------

                                        virG
  pSYN12274  (8501) AGGCTGCGCCGCAAACTTGAGGCAGATCCGTCAAGCCCTCAACTGATAAA
5307 insert         ---------------------------------------------------

                                        virG
  pSYN12274  (8551) AACAGCAAGAGGTGCCGGTTATTTCTTTGACGCGGACGTGCAGGTTTCGC
5307 insert         ---------------------------------------------------

                                virG
  pSYN12274  (8601) ACGGGGGGACGATGGCAGCCTGAGCCAATTCCCAGATCCCCGAGGAATCG
5307 insert         ---------------------------------------------------

                                         repA
  pSYN12274  (8651) GCGTGAGCGGTCGCAAACCATCCGGCCCGGTACAAATCGGCGCGGCGCTG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (8701) GGTGATGACCTGGTGGAGAAGTTGAAGGCCGCGCAGGCCGCCCAGCGGCA
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (8751) ACGCATCGAGGCAGAAGCACGCCCCGGTGAATCGTGGCAAGCGGCCGCTG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (8801) ATCGAATCCGCAAAGAATCCCGGCAACCGCCGGCAGCCGGTGCGCCGTCG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (8851) ATTAGGAAGCCGCCCAAGGGCGACGAGCAACCAGATTTTTTCGTTCCGAT
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (8901) GCTCTATGACGTGGGCACCCGCGATAGTCGCAGCATCATGGACGTGGCCG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (8951) TTTTCCGTCTGTCGAAGCGTGACCGACGAGCTGGCGAGGTGATCCGCTAC
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9001) GAGCTTCCAGACGGGCACGTAGAGGTTTCCGCAGGGCCGGCCGGCATGGC
5307 insert         ---------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        repA
  pSYN12274  (9051) CAGTGTGTGGGATTACGACCTGGTACTGATGGCGGTTTCCCATCTAACCG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9101) AATCCATGAACCGATACCGGGAAGGGAAGGGAGACAAGCCCGGCCGCGTG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9151) TTCCGTCCACACGTTGCGGACGTACTCAAGTTCTGCCGGCGAGCCGATGG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9201) CGGAAAGCAGAAAGACGACCTGGTAGAAACCTGCATTCGGTTAAACACCA
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9251) CGCACGTTGCCATGCAGCGTACGAAGAAGGCCAAGAACGGCCGCCTGGTG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9301) ACGGTATCCGAGGGTGAAGCCTTGATTAGCCGCTACAAGATCGTAAAGAG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9351) CGAAACCGGGCGGCCGGAGTACATCGAGATCGAGCTAGCTGATTGGATGT
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9401) ACCGCGAGATCACAGAAGGCAAGAACCCGGACGTGCTGACGGTTCACCCC
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9451) GATTACTTTTTGATCGATCCCGGCATCGGCCGTTTTCTCTACCGCCTGGC
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9501) ACGCCGCGCCGCAGGCAAGGCAGAAGCCAGATGGTTGTTCAAGACGATCT
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9551) ACGAACGCAGTGGCAGCGCCGGAGAGTTCAAGAAGTTCTGTTTCACCGTG
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9601) CGCAAGCTGATCGGGTCAAATGACCTGCCGGAGTACGATTTGAAGGAGGA
5307 insert         ---------------------------------------------------

                                        repA
  pSYN12274  (9651) GGCGGGGCAGGCTGGCCCGATCCTAGTCATGCGCTACCGCAACCTGATCG
5307 insert         ---------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                        repA
  pSYN12274  (9701) AGGGCGAAGCATCCGCCGGTTCCTAATGTACGGAGCAGATGCTAGGGCAA
5307 insert         --------------------------------------------------

                                                VS1 ori
  pSYN12274  (9751) ATTGCCCTAGCAGGGGAAAAAGGTCGAAAAGGTCTCTTTCCTGTGGATAG
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274  (9801) CACGTACATTGGGAACCCAAAGCCGTACATTGGGAACCGGAACCCGTACA
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274  (9851) TTGGGAACCCAAAGCCGTACATTGGGAACCGGTCACACATGTAAGTGACT
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274  (9901) GATATAAAAGAGAAAAAAGGCGATTTTTCCGCCTAAAACTCTTTAAAACT
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274  (9951) TATTAAAACTCTTAAAACCCGCCTGGCCTGTGCATAACTGTCTGGCCAGC
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274 (10001) GCACAGCCGAAGAGCTGCAAAAAGCGCCTACCCTTCGGTCGCTGCGCTCC
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274 (10051) CTACGCCCCGCCGCTTCGCGTCGGCCTATCGCGGCCGCTGGCCGCTCAAA
5307 insert         --------------------------------------------------

                                  VS1 ori
  pSYN12274 (10101) AATGGCTGGCCTACGGCCAGGCAATCTACCAGGGCGCGGACAAGCCGCGC
5307 insert         --------------------------------------------------

                          VS1 ori
  pSYN12274 (10151) CGTCGCCACTCGACCGCCGGCGCTGAGGTCTGCCTCGTGAAGAAGGTGTT
5307 insert         --------------------------------------------------


  pSYN12274 (10201) GCTGACTCATACCAGGCCTGAATCGCCCCATCATCCAGCCAGAAAGTGAG
5307 insert         --------------------------------------------------


  pSYN12274 (10251) GGAGCCACGGTTGATGAGAGCTTTGTTGTAGGTGGACCAGTTGGTGATTT
5307 insert         --------------------------------------------------


  pSYN12274 (10301) TGAACTTTTGCTTTGCCACGGAACGGTCTGCGTTGTCGGGAAGATGCGTG
5307 insert         --------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
  pSYN12274 (10351) ATCTGATCCTTCAACTCAGCAAAAGTTCGATTTATTCAACAAAGCCGCCG
5307 insert         --------------------------------------------------


  pSYN12274 (10401) TCCCGTCAAGTCAGCGTAATGCTCTGCCAGTGTTACAACCAATTAACCAA
5307 insert         --------------------------------------------------


  pSYN12274 (10451) TTCTGATTAGAAAAACTCATCGAGCATCAAATGAAACTGCAATTTATTCA
5307 insert         --------------------------------------------------


  pSYN12274 (10501) TATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAA
5307 insert         --------------------------------------------------


  pSYN12274 (10551) GGAGAAAACTCACCGAGGCAGTTCCATAGGATGGCAAGATCCTGGTATCG
5307 insert         --------------------------------------------------


  pSYN12274 (10601) GTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTAATTTCCCCT
5307 insert         --------------------------------------------------


  pSYN12274 (10651) CGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAA
5307 insert         --------------------------------------------------


  pSYN12274 (10701) TCCGGTGAGAATGGCAAAAGCTCTGCATTAATGAATCGGCCAACGCGCGG
5307 insert         --------------------------------------------------


  pSYN12274 (10751) GGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGAC
5307 insert         --------------------------------------------------


  pSYN12274 (10801) TCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAA
5307 insert         --------------------------------------------------

                                    ColE1 ori
                    ____________________________________________________
  pSYN12274 (10851) GGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACA
5307 insert         --------------------------------------------------

                                    ColE1 ori
                    ____________________________________________________
  pSYN12274 (10901) TGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTG
5307 insert         --------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        ColE1 ori
  pSYN12274 (10951) CTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCG
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11001) ACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGG
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11051) CGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCG
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11101) CTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTC
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11151) TCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCA
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11201) AGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTA
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11251) TCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCC
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11301) ACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCG
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11351) GTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGA
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11401) ACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAG
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11451) AGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTT
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11501) TTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAA
5307 insert         --------------------------------------------------

                                        ColE1 ori
  pSYN12274 (11551) GATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTC
5307 insert         --------------------------------------------------
```

Figure 4. Alignment of the 5307 maize insert with the transformation plasmid, pSYN12274 *(Continued)*

```
                                        ColE1 ori
  pSYN12274 (11601) ACGTTAAGGGATTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGA
5307 insert         --------------------------------------------------

                    ColE1 ori
  pSYN12274 (11651) TCCTTTTGATCCGGAATTAATTCCTGTGGTTGGCATGCACATACAAATGG
5307 insert         --------------------------------------------------


  pSYN12274 (11701) ACGAACGGATAAACCTTTTCACGCCCTTTTAAATATCCGATTATTCTAAT
5307 insert         --------------------------------------------------


  pSYN12274 (11751) AAACGCTCTTTTCTCTTAG
5307 insert         -------------------
```