98-238-01p

AgrEvo

August 24, 1998

Ms. Rebecca Bech
Director, Scientific Services
USDA, APHIS, PPQ, SS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Re: **Request for Extension of a Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events (96-068-1p)**

Dear Ms. Bech:

AgrEvo USA Company is submitting an Application for an Extension of the Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) for Glufosinate Resistant Soybean (GRS) Events previously granted under 96-0681p. The new event is called **GU262.** This event was transformed in the same manner and using the same plasmid as were the events in the previous petition. The data submitted supports the contention that this event exhibits the same properties as the previously approved events. Termination reports from all relevant field trials are enclosed.

Enclosed are two copies of the petition extension for GU262. The enclosed request does not contain any confidential business information.

AgrEvo will request permission in January 1999 to conduct a field trial of event GU262 in Japan during the 1999 season. The publication in the Federal Register of a Determination of Nonregulated Status for the event being tested greatly facilitates the process for obtaining permission to conduct a field test in Japan. Therefore, it would be helpful if the petition extension for GU262 were processed in a timely fashion.

Please contact me at (302) 892-3155 if you have any questions concerning our petition.

Best Regards,

Sally Van Wert

Sally Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology

Enclosures (2)

AgrEvo USA Company
Little Falls Centre One, 2711 Centerville Road, Wilmington, DE 19808, Telephone: (302) 892-3000, Fax: (302) 892-3013

8/26/98

98-238-01p

Application for an Extension of the Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events (96-068-1p):

Event GU262

The undersigned submits this petition under 7 CFR 340.6 to request that the Director, Scientific Services, make a determination that the article should not be regulated under 7 CFR 340.

Submitted by:

Sally Van Wert

Sally Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology

AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808
Telephone: 302-892-3155
FAX: 302-892-3099

August 24, 1998

Contains No Confidential Business Information

8/26/98

Summary

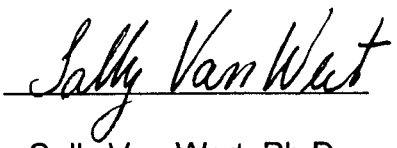
Event GU262 has been field tested by Pioneer Hi-Bred International, Inc. and AgrEvo USA Company, since 1996 in the primary soybean growing regions of the southern United States. These tests have occurred at approximately 16 sites under field release authorizations granted by APHIS (USDA authorizations: 96-134-03N, 96-184-01N, 97-038-02N, 97-038-03N, 98-040-07N, 98-040-08N, 98-040-09N, 98-071-23N, 98-078-04N, 98-078-23N, 98-125-03N). Data collected from these trials, laboratory analyses, reports, and literature references presented herein demonstrate that Glufosinate Resistant Soybean (GRS) event GU262: 1) exhibits no plant pathogenic properties; 2) is no more likely to become a weed than non-modified soybean; 3) is unlikely to increase the weediness potential of any other cultivated plant or native wild species; 4) does not cause damage to processed agricultural commodities; and 5) is unlikely to harm other organisms that are beneficial to agriculture. Transformation event GU262 has also been field tested in Chile and Argentina in 1997/98.

Primary transformation event GU262 has been crossed with Pioneer's proprietary lines. The primary transformation event and its progeny are collectively referred to as GRS in this petition extension.

AgrEvo USA Company requests a determination from APHIS that GRS transformation event GU262 and any progeny derived from crosses of this event with traditional soybean varieties, and any progeny derived from crosses of this event with transgenic soybean varieties that have also received a determination of nonregulated status, no longer be considered regulated articles under 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.



Sally Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology

AgrEvo USA Company
Little Falls Centre One
2711 Centerville Road
Wilmington, DE 19808
Telephone: 302-892-3155
FAX: 302-892-3099

ACRONYMS AND SCIENTIFIC TERMS

bla - ß-lactamase gene, ampicillin resistance gene
ELISA - enzyme linked immunosorbent assay
GA - glufosinate-ammonium
GRS - glufosinate resistant soybean
GS – glutamine synthetase
PAT - phosphinothricin acetyltransferase
pat - phosphinothricin acetyltransferase gene (origin *S. viridochromogenes*)
PCR - polymerase chain reaction

TABLE OF CONTENTS

A. Statement of Grounds for Nonregulated Status

LIST OF TABLES

LIST OF FIGURES

Statement of Grounds for Nonregulated Status

I. Rationale for Submission of Request for Extension

There are no changes in rationale from the previously approved petition number 96-068-01p. The specific differences between Glufosinate Resistant Soybean Event (GRS) GU262 and the events in the previous petition are discussed in the appropriate sections. The new event to be considered under this extension is GU262. In notifications and termination reports event GU262 also has been referred to or designated as PHI2-GU262.

II. The Soybean Family

There are no changes from the previously approved petition submission.

III. The Transformation System, Plasmid and Parent Line Used

The GRS transformation event GU262 was transformed in the same manner and using the same plasmid (pB2/35AcK) as were GRS events A2704-12, A2704-21 and A5547-35 (see Appendix and petition 96-068-01p for details) and A5547-127 (see petition extension 98-014-10p for details). The plasmid has been also referred to or designated as PHP6516 and pWRG5143 in notifications. A Pioneer Hi-Bred International, Inc. (Johnston , IA) *Glycine max* cultivar coded PHI2 was used for transformation, resulting in primary transformation event GU262. Prior to transformation the vector was digested with *Dra*I to disrupt the coding sequence of the ß-lactamase (*bla*) gene. This restriction endonuclease excises a 692bp DNA fragment containing 3' sequences from the *bla* gene and a 3365 bp fragment containing a synthetic *pat* gene fused to the *35S* promoter and terminator of Cauliflower Mosaic Virus and 5' sequences of the *bla* gene. Previous GRS events were generated using the vector digested with *Pvu*I (see petition 96-068-01p for details), which also disrupts the *bla* gene.

The parent line PHI2 is a maturity group V cultivar of the moderately narrow canopy type, with white flowers, gray pubescence, buff hila and tan podwall color. It is a high yielding determinant variety with moderate to good tolerance to southern root-knot nematode (*Meloidogyne javanica*) and frogeye leaf spot (*Cercospora sojina*). This cultivar is a long season type that performs optimally between 32-35 degrees north latitude.

Transformation event GU262 has been crossed with elite breeding lines. As was the strategy for the previous GRS events, the commercialization strategy for

GU262 is to use traditional backcrossing and breeding to transfer the glufosinate-ammonium (GA) resistance locus from the transformation event to a wide range of varieties with a wide range of maturities. Resistance to GA is conferred by expression of the phosphinothricin acetyltransferase (*pat*) gene.

IV. Genetic Characterization of Event GU262

A. Description, History and Mendelian Inheritance

Event GU262 was evaluated in the field in 1996 at 2 sites, in 1997 at 3 sites and in 1998 at 12 sites under authorizations granted by APHIS (USDA authorizations: 96-134-03N, 96-184-01N, 97-038-02N, 97-038-03N, 98-040-07N, 98-040-08N, 98-040-09N, 98-071-23N, 98-078-04N, 98-078-28N, 98-125-03N). The purpose of the trials was to increase seed, advance generations, demonstrate the agronomic performance, to evaluate segregation ratios and/or to collect samples for analytical work regarding the composition and nutrient and transgene product in this additional event.

The *pat* locus has been inherited in the Mendelian fashion, fitting the prediction for a single locus, in GU262 homozygotes for a few generations. To transfer the *pat* gene from this transformation event to commercially viable material the original hemizygous transformed plant was self-pollinated. The resultant progeny were evaluated In Mississippi in the 1996 growing season and resulted in T1 progeny segregating in a 3:1 fashion with respect to glufosinate resistance (Table 1). Seed from all surviving plants from the event were advanced in winter nursery to accumulate additional data. The T1:2 plant rows were grown in Puerto Rico. Expected phenotypes for each row would be of two types (entire rows resistant : partial rows resistant) dependent upon whether the T1 plant was homozygous or heterozygous for the *pat* locus (Table 1). The progeny from fully resistant rows were homozygous for the *pat* locus while those from partially resistant rows were heterogeneous with respect for the locus. Homozygous plants were those from which all progeny from the 2nd self-pollination (grown in Puerto Rico) were unharmed by GA.

If the *pat* locus is stable, then all progeny should be resistant to GA in subsequent generations. This was evaluated during subsequent generations and found to be true. The commercial lines were selected from these homozygous plants.

To confirm the expected Mendelian segregation of 1:1 for the *pat* locus, crosses between a single GU262 T1 resistant plant and a non-transgenic PHI2 plant were made in the Pioneer greenhouse facilities at Johnston, Iowa, resulting in F1 seed. The T1 plant used as the male was determined to be heterozygous for the *pat* gene from a progeny test. The F1 seed was planted in the greenhouse and

Table 1. Segregation Data for Individuals and Rows of Progeny of Self-pollinated or Cross-Pollinated Event GU262

Comparison	Progeny [a]	Resistant Plants	Sensitive Plants	Expected Ratio	χ^{2b}
plants	T1	36	14	3:1	0.077
Comparison	**Progeny [a]**	**Resistant Plants**	**Sensitive Plants**	**Expected Ratio**	**χ^{2b}**
row 1	T1:2	127	58	all resistant or 3:1	2.99
row 3	T1:2	115	0	all resistant or 3:1	homozygous
row 6	T1:2	108	50	all resistant or 3:1	2.79
row 14	T1:2	115	38	all resistant or 3:1	0.0
row 16	T1:2	182	0	all resistant or 3:1	homozygous
row 17	T1:2	175	1	all resistant or 3:1	homozygous
row 19	T1:2	117	0	all resistant or 3:1	homozygous
row 25	T1:2	96	40	all resistant or 3:1	1.06
row 26	T1:2	11	33	all resistant or 3:1	0.25
row 30	T1:2	112	26	all resistant or 3:1	2.09
row 31	T1:2	108	47	all resistant or 3:1	1.76
row 32	T1:2	112	35	all resistant or 3:1	0.08
row 34	T1:2	137	0	all resistant or 3:1	homozygous
row 35	T1:2	112	23	all resistant or 3:1	3.42
row 36	T1:2	151	0	all resistant or 3:1	homozygous
row 37	T1:2	118	31	all resistant or 3:1	1.05
row 39	T1:2	103	0	all resistant or 3:1	homozygous
row 40	T1:2	106	27	all resistant or 3:1	1.17
row 41	T1:2	105	37	all resistant or 3:1	0.06
row 42	T1:2	127	28	all resistant or 3:1	2.98
row 47	T1:2	76	1	all resistant or 3:1	homozygous
row 48	T1:2	60	22	all resistant or 3:1	0.11
Comparison	**Progeny [a]**	**Resistant Plants**	**Sensitive Plants**	**Expected Ratio**	**χ^{2b}**
plants	F1	11	9	1:1	0.036

[a] T1 = segregation of individual progeny from self-pollination of original To plant; T1:2 = segregation of entire versus partially resistant rows derived from resistant T1 plants; F1 = segregation of individual progeny from the cross between a single T1 resistant plant and a non-transformed PHI2 plant.

[b] No significant difference (p=0.05) for the Chi square goodness-of-fit test for hypothesis of either 1:1 or 3:1 segregation. (Significance at p=0.05 for $\chi^2 \geq 3.84$, df = 1). * Significant difference found.

the progeny were sprayed with glufosinate. The segregation ratio fits a single-dominant gene model (Table 1).

B. DNA Analysis of Event GU262

To determine the nature and number of *pat* and *bla* gene insertions which occur in transformation event GU262, Southern hybridization and Polymerase Chain Reaction (PCR) analysis were used. When transforming a plant with restriction digested or intact, circular vector DNA there is no way to predict at which site or sites on the vector recombination will initiate. We have therefore used Southern blot and PCR analyses to examine the integrity of the inserted vector in GRS transformation events. Event GU262 differs in the copy number and extend of integrated DNA from the events that were the subject of petition 96-068-01p and petition extension 98-014-01p (see Appendix).

In the experiments restriction digested genomic DNA from transgenic plants homozygous for the integrated DNA were run in parallel with a digested genomic DNA from a nontransgenic PHI2 plant, supplemented with approximately 2 copies of digested transforming plasmid. The determination of the integrated copies is deduced from analyzing all obtained Southern blot data. PCR analysis was used to confirm the orientation of the inserted copies.

Several aliquots of event GU262 DNA were digested with restriction enzymes. See Figures 1 and 2 to locate restriction sites in pB2/35SAck. After separation of the DNA by electrophoresis, the DNA was transferred to a nylon membrane and hybridized with a ^{32}P-labeled Polymerase Chain Reaction (PCR) generated *pat* gene fragment (Figures 3), with ^{32}P-labeled *bla* gene fragments (Figures 4 and 5), or with ^{32}P-labeled ori sequence fragments (Figure 6). The primers used to generate the target sequences are between 31 and 19 nucleotides in length and were primarily located internal to the target sequences (Figure 2). Lanes contain approximately 10 µg of restricted DNA. The amount of restricted pB2/35SAck in positive control lanes is equivalent to 2.0 copy of the plasmid integrated in 10 µg of soybean DNA. The probed membranes were visualized by autoradiography. The same membranes were striped and rehybridized with different probes. Electronic scans of the autoradiographs are presented in this document.

The primer pairs used to generate probes and their target sequence are listed in Table 2. The locations of the primers are shown in Figure 2. The hybridizing fragments expected and observed when using the probes are listed in Table 3. The sizes of some hybridizing fragments can be predicted by the location of restriction enzyme cleavage sites internal to the inserted vector. Those hybridizing fragments whose sizes cannot be predicted result from cleavage in

the integrated vector and in the adjacent plant DNA. A schematic summary drawing of the insert of soybean event GU262 is presented in Figure 7.

Table 2. Primer Pairs and Target Sequences

Probe	Primer-pair	Probe size (bp)	pB2/35SAcK – EcoRI/DraI digest Expected hybridizing fragments (bp)
pat	MDB403-MDB404	623	1329
3' *bla*	MDB402-MDB538	666	692
5' *bla* + vector	MDB539-MDB540	821	867
ori + vector	MDB436-MDB537	1068	1169

1. *pat* Gene

The results obtained with SphI, HindIII and EcoRV digested GU262 genomic DNA indicate two copies of the *pat* gene are inserted in the genome. The two *pat* gene copies are intact since the expected 489 bp and 315 bp BamHI fragments, the 2963 bp BspHI fragment and the 1329 bp EcoRI fragment are observed (Figure 3, lanes 5, 6 and 10). The two *pat* copies reside on one DraI fragment (Figure 3, lane 3). The hybridization results obtained with NcoI digested GU262 genomic DNA are due to partial digestion of the genomic DNA.

2. *bla* Gene

Two probes were used to analyze the integration of *bla* sequences into the genome of soybean event GU262 plants (Figure 2). The 3' *bla* (666 bp) is homologous to *bla* sequences found between 3003 bp and 3646 bp of pB2/35SAcK. This probe basically targets the DraI fragment that contains the majority of the *bla* gene. The 5' *bla* + vector probe (821 bp) is homologous to pB2/35SAcK sequences from position 451 until position 3706. It contains 5' *bla* sequences and pB2/35SAcK vector sequences. No hybridizing fragments were observed in lanes containing GU262 DNA when the 3' *bla* probe was used (Figure 4). The 3' *bla* probe hybridized to the expected fragments of the DNA positive controls (Figure 4, lanes 13 and 14), showing that the hybridization was performed under conditions allowing hybridization of the probe with target sequences. The Southern blot hybridization results obtained with the 3' *bla* probe indicate that this region of the *bla* gene is not integrated into the soybean genome.

Table 3. Observed and Expected[a] Hybridizing Fragments in Southern Blots of GU262 DNA

Digest	*pat*		*3′ bla*		*5′ bla* + vector		ori + vector		pB2/35SAcK
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected	Observed
None	**> 20 Kb**	> 20 Kb	**None**	- - -	**> 20 Kb**	> 20 Kb	**> 20 Kb**	> 20 Kb	**> 20 Kb**
DraI	**14000**	> 3365	**None**	> 666	**14000**	> 3365	**14000**	> 3365	**14000**
EcoRV	**> 20 Kb** **7200**	> 1875 181	**None**	> 666	**7200** **2300**	> 1309	**> 20 Kb** **7200**	> 1875	**>20 Kb** **7200, 2300**
BamHI	**489** **315**	489 315	**None**	> 666	**6800** **2500**	> 1413	**> 20 Kb** **6800**	> 1148	**> 20 Kb** **6800, 2500, 489, 315**
BspHI	**2963**	2963	**None**	> 666	(5500), **2963**	2963, 105	**2963**	2963	**5500, 2963**
SphI	**8500** **4500**	> 1990	**None**	> 666	**8500** **4500**	> 1990	**2700** **1400**	> 1120	**8500,4500, 2700, 1400**
HindIII	**7400** **6400**	> 2247	**None**	> 666	**7400** **6400**	> 2247	**7400** **1800**	> 1118	**7400, 6400, 1800**
HindIII DraI	**7400** **3500**	> 2247	**None**	> 666	**7400** **3500**	> 2247	**7400** **1000**	> 1118	**7400, 3500, 1000**
EcoRI	**1329**	1329	**None**	> 666	**6200** **1500**	> 1309	**7800** **6200**	> 1875	**7800, 6200, 1500, 1329**
NcoII	**8000,6400, 5500,3800, 2800**	> 2492	**None**	> 666	**8000, 6400, 5500, 4500, 3500, 1500**	> 873	**8000, 6400, 5500, 3800, 2800**	> 2492	**8000, 6400, 5500, 4500, 3800, 3500, 2800, 1500**

[a] Expected fragment sizes for 1 copy of inserted vector.

The Southern blot hybridization results obtained when EcoRV, BamHI, SphI, HindIII and EcoRI digested GU262 DNAs were probed with the 5' *bla* + vector probe indicate the insertion of two copies of the 5' *bla* + vector sequences (Figure 5, lanes 4, 5, 7, 8 and 10). With BspHI digested genomic GU262 DNA two fragments were observed: an internal 2963 bp fragment and a somewhat weaker 5500 bp fragment. This 5500 bp fragment represents the junction between the two inserted plasmid copies (see Figure 7 and summary below). Again, the hybridization result obtained with NcoI digested GU262 genomic DNA is due to partial digestion of the genomic DNA.

3. Ori Sequence

The Southern blot hybridization results with the ori probe indicate the insertion of two copies of the origin of replication with EcoRV, BamHI, SphI, HindIII and EcoRI digested GU262 DNA (Figure 6, lanes 4, 5, 7, 8 and 10).

4. Summary

Southern blot analysis, using the *pat* probe, revealed the presence of an 8500 bp and a 4500 bp SphI hybridizing fragment. This enzyme has an unique restriction recognition site in the transforming pB2/35SAcK plasmid. Mapping of the complete insert showed the 8500 bp SphI fragment represents the junction fragment between plant DNA sequences and transgenic DNA sequences of one integrated plasmid copy. The 4500 bp SphI fragment was shown to represent the junction fragment between plant DNA sequences and transgenic sequences of the second plasmid copy (Figure 7).

PCR analysis confirmed the 'Head-to-Tail' insertion of two copies of the transforming plasmid: using MDB483 as a forward primer and MDB482 as a reverse primer (Figure 7), a 5 kb fragment was amplified (data not shown). Digestion of this amplified fragment with the SphI restriction enzyme yielded a 1900 bp and 3100 bp fragment (data not shown) confirming the presence of a SphI site in the junction DNA. This finding was in complete agreement with Southern blot results obtained with SphI digested GU262 genomic DNA.

In summary, Southern blot analysis of soybean event GU262 indicates a 'Head-to-Tail' insertion of two copies of the transforming DNA. Results indicate that two copies of the *pat* gene cassette and ori sequences are integrated into the plant genome. Hybridization results obtained with the different *bla* probes indicate that there are no 3' *bla* sequences present in GU262 but that there are two copies of the 5' *bla* sequences integrated. Southern blot hybridization with the total plasmid pB2/35SAcK yielded an inventory of all hybridizing fragments for the different digests (data not shown, results in Table 3). The obtained fingerprints

represent the sum of the fingerprints obtained with the probes representing the different components of the transforming plasmid.

All probes were specific to the introduced sequences in event GU262 since no hybridization was seen with nontransgenic soybean (see Figures 3-6, lane 12). With every probe used the DNA positive controls yielded fragments of the expected size (see Table 2 and Figures 2-6, lanes 13 and 14), indicating that all hybridizations were performed under conditions allowing hybridization of the probe with target sequences. A schematic summary drawing of the insert of soybean event GU262 is presented in Figure 7.

Figure 1. Vector Map of pB2/35SAck

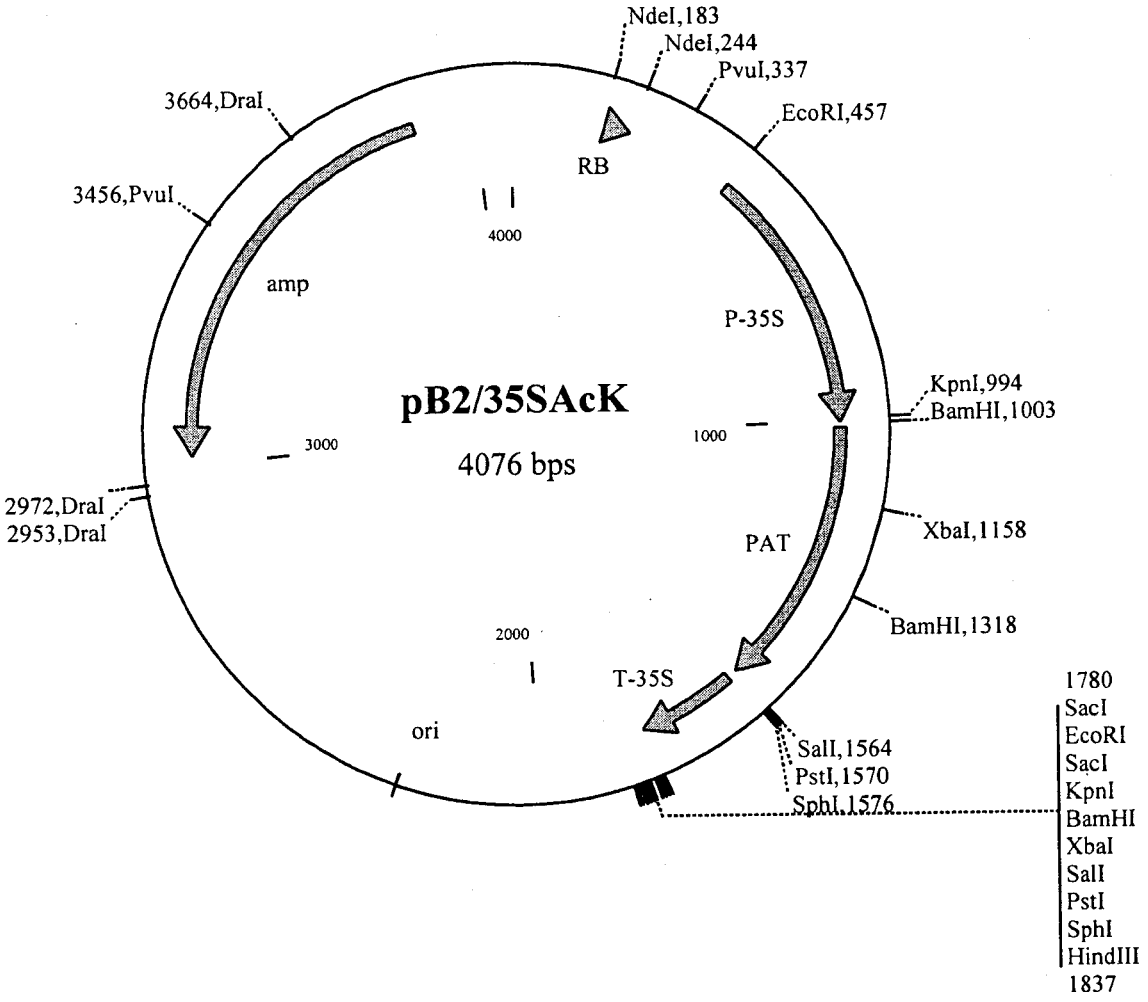


Figure 2. Location of Primers on pB2/35SAcK

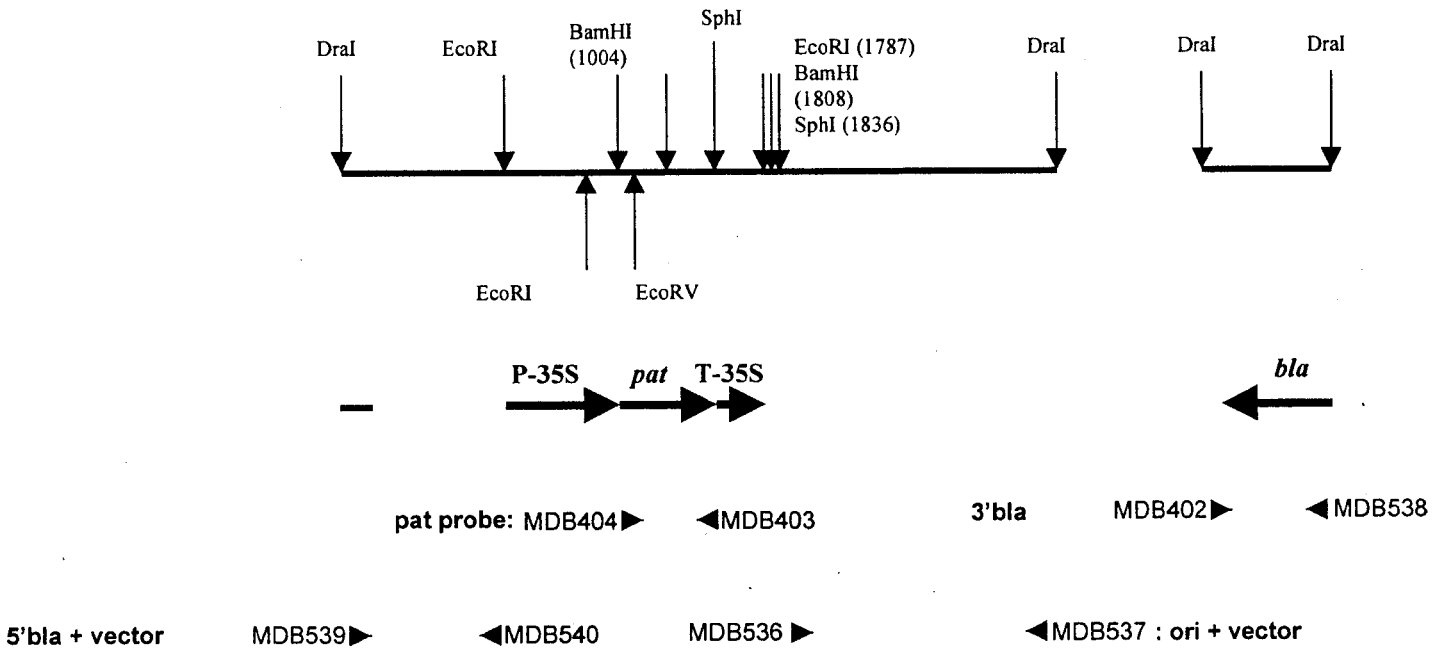


Figure 3. Southern Blot Analysis: Soybean Event GU262 - *pat* probe. DNA was isolated from GRS event GU262 and the nontransgenic parent line PHI2-NT. DNAs (10 μg) were digested with the indicated restriction enzymes. The *pat* fragment (623 bp)(see Figure 2) was used as probe. Lane 1. MW-marker. Lane 2. GU262: undigested. Lane 3. GU262: DraI digest. Lane 4. GU262: EcoRV digest. Lane 5. GU262: BamHI digest. Lane 6. GU262: BspHI digest. Lane 7. GU262: SphI digest. Lane 8. GU262: HindIII digest. Lane 9. GU262: HindIII/DraI digest. Lane 10. GU262: EcoRI digest. Lane 11. GU262: NcoI digest. Lane 12. PHI2-NT: HindIII digest. Lane13. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with PHI2-NT: HindIII digest). Lane 14 pB2/35SAcK plasmid: HindIII digest (supplemented with PHI2-NT: HindIII digest). The amount of restricted pB2/35SAck in lanes 13 and 14 is equivalent to 2.0 copy of the plasmid integrated in 10 μg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

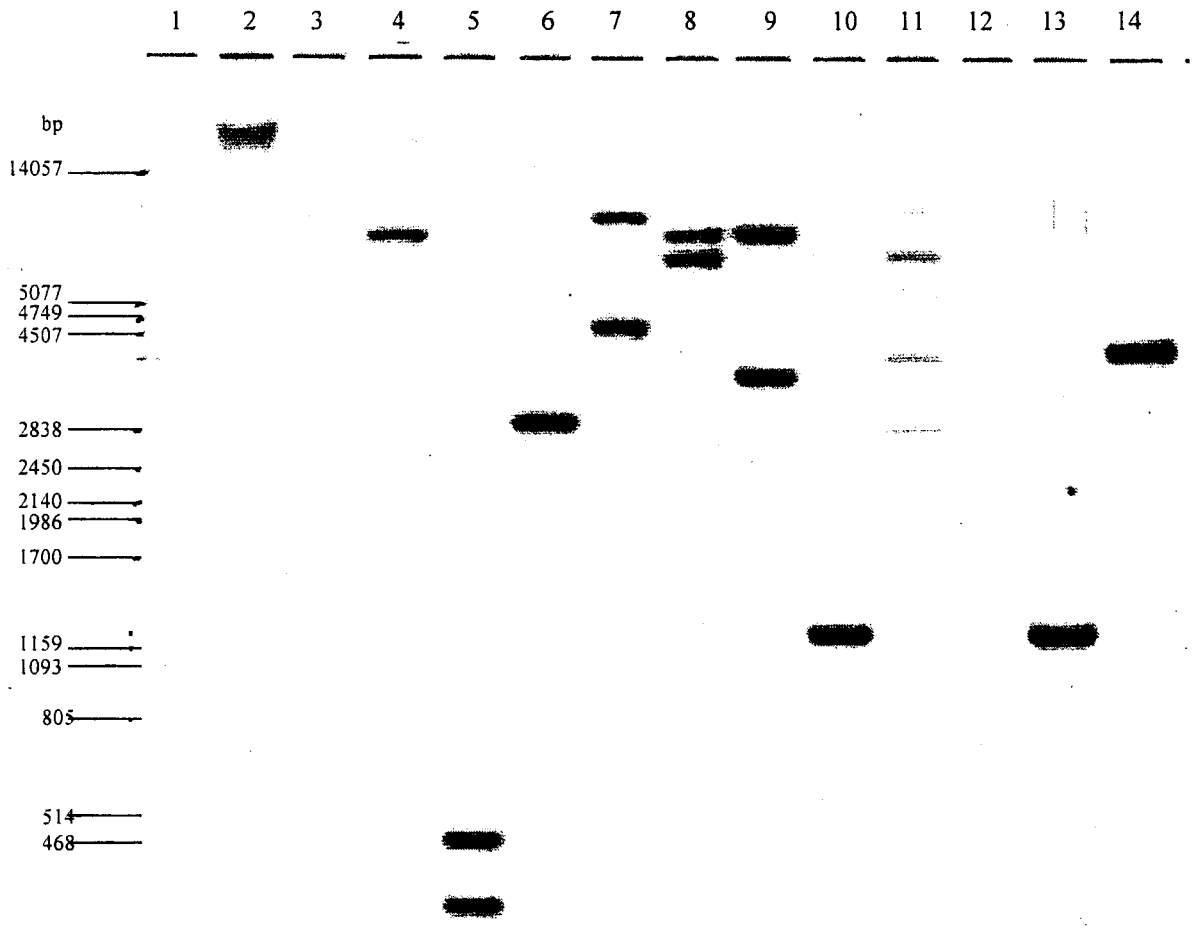


Figure 4. Southern Blot Analysis: Soybean Event GU262 – 3' *bla* probe. DNA was isolated from GRS event GU262 and the nontransgenic parent line PHI2-NT. DNAs (10 µg) were digested with the indicated restriction enzymes. The 3' *bla* fragment (666 bp)(see Figure 2) was used as probe. Lane 1. MW-marker. Lane 2. GU262: undigested. Lane 3. GU262: DraI digest. Lane 4. GU262: EcoRV digest. Lane 5. GU262: BamHI digest. Lane 6. GU262: BspHI digest. Lane 7. GU262: SphI digest. Lane 8. GU262: HindIII digest. Lane 9. GU262: HindIII/DraI digest. Lane 10. GU262: EcoRI digest. Lane 11. GU262: NcoI digest. Lane 12. PHI2-NT: HindIII digest. Lane13. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with PHI2-NT: HindIII digest). Lane 14 pB2/35SAcK plasmid: HindIII digest (supplemented with PHI2-NT: HindIII digest). The amount of restricted pB2/35SAck in lanes 13 and 14 is equivalent to 2.0 copy of the plasmid integrated in 10 µg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

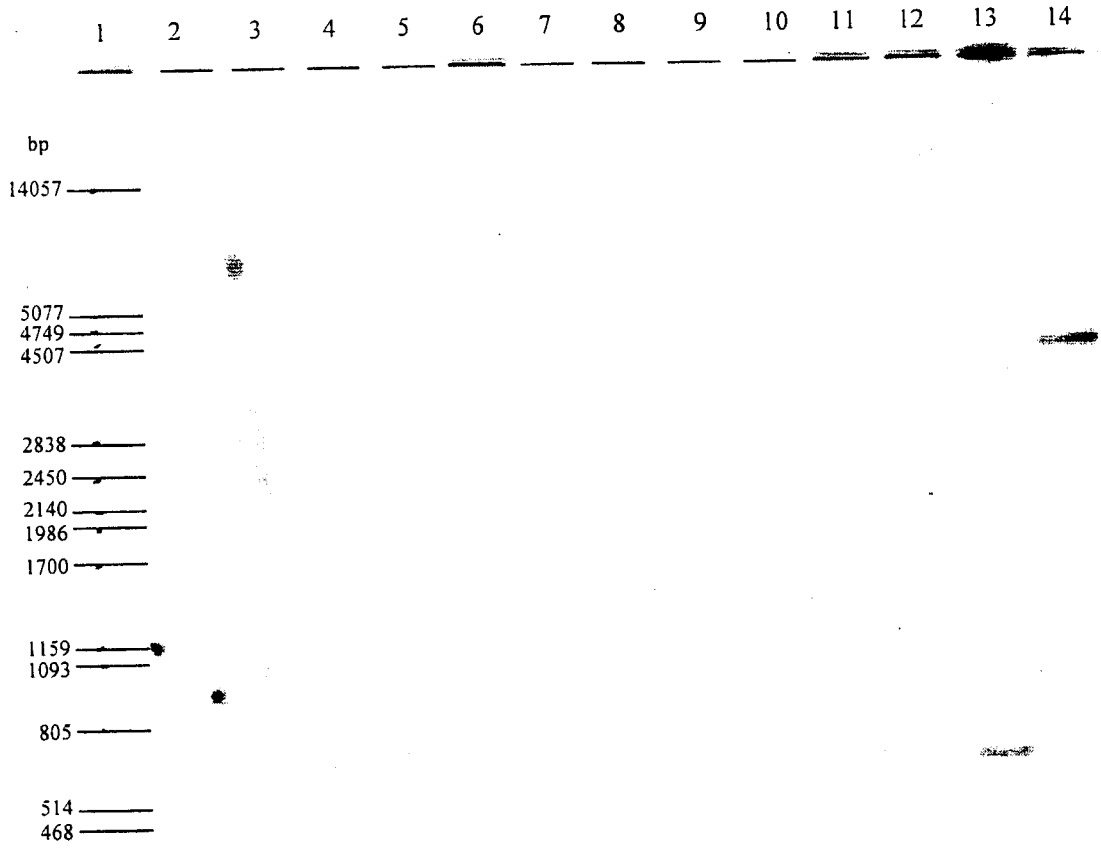


Figure 5. Southern Blot Analysis: Soybean Event GU262 – 5' *bla* probe. DNA was isolated from GRS event GU262 and the nontransgenic parent line PHI2-NT. DNAs (10 μg) were digested with the indicated restriction enzymes. The 5' *bla* + vector fragment (821 bp)(see Figure 2) was used as probe. Lane 1. MW-marker. Lane 2. GU262: undigested. Lane 3. GU262: DraI digest. Lane 4. GU262: EcoRV digest. Lane 5. GU262: BamHI digest. Lane 6. GU262: BspHI digest. Lane 7. GU262: SphI digest. Lane 8. GU262: HindIII digest. Lane 9. GU262: HindIII/DraI digest. Lane 10. GU262: EcoRI digest. Lane 11. GU262: NcoI digest. Lane 12. PHI2-NT: HindIII digest. Lane13. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with PHI2-NT: HindIII digest). Lane 14 pB2/35SAcK plasmid: HindIII digest (supplemented with PHI2-NT: HindIII digest). The amount of restricted pB2/35SAck in lanes 13 and 14 is equivalent to 2.0 copy of the plasmid integrated in 10 μg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

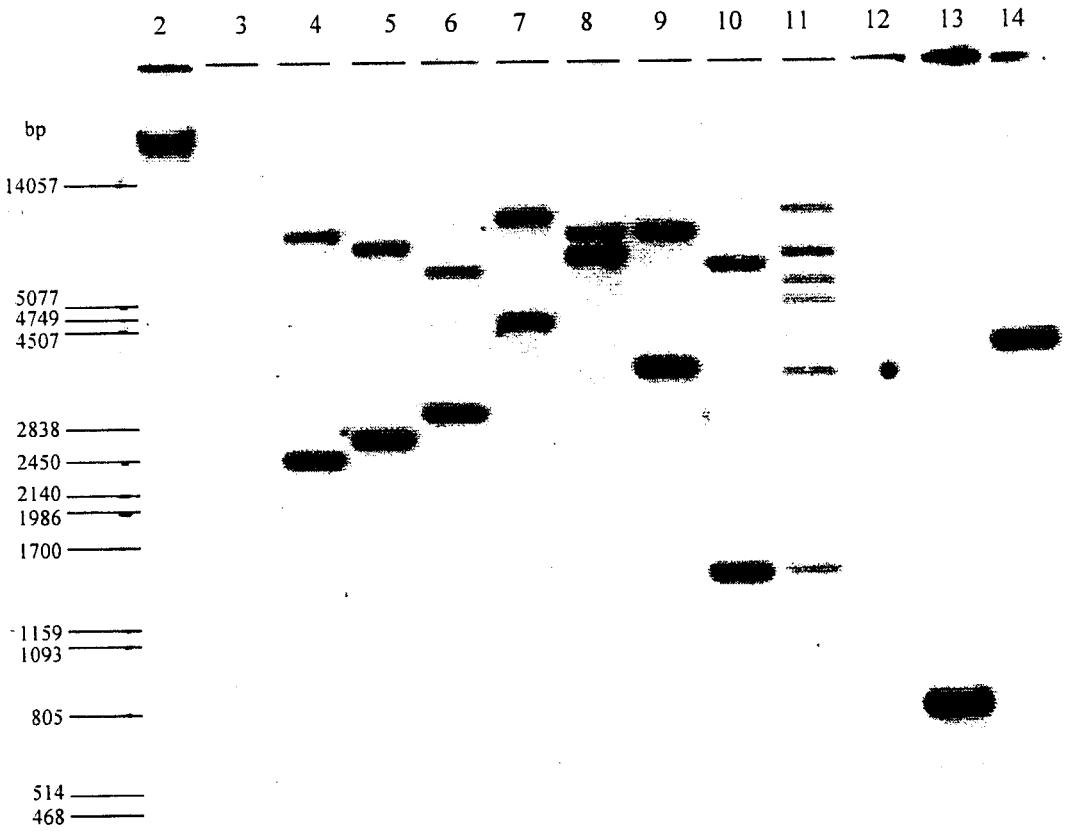


Figure 6. Southern Blot Analysis: Soybean Event GU262 – ori probe. DNA was isolated from GRS event GU262 and the nontransgenic parent line PHI2-NT. DNAs (10 μg) were digested with the indicated restriction enzymes. The ori + vector fragment (1068 bp)(see Figure 2) was used as probe. Lane 1. MW-marker. Lane 2. GU262: undigested. Lane 3. GU262: DraI digest. Lane 4. GU262: EcoRV digest. Lane 5. GU262: BamHI digest. Lane 6. GU262: BspHI digest. Lane 7. GU262: SphI digest. Lane 8. GU262: HindIII digest. Lane 9. GU262: HindIII/DraI digest. Lane 10. GU262: EcoRI digest. Lane 11. GU262: NcoI digest. Lane 12. PHI2-NT: HindIII digest. Lane13. pB2/35SAcK plasmid: EcoRI/DraI digest (supplemented with PHI2-NT: HindIII digest). Lane 14 pB2/35SAcK plasmid: HindIII digest (supplemented with PHI2-NT: HindIII digest). The amount of restricted pB2/35SAck in lanes 13 and 14 is equivalent to 2.0 copy of the plasmid integrated in 10 μg of soybean DNA. MW marker (λ DNA digested with PstI) sizes given in base pairs.

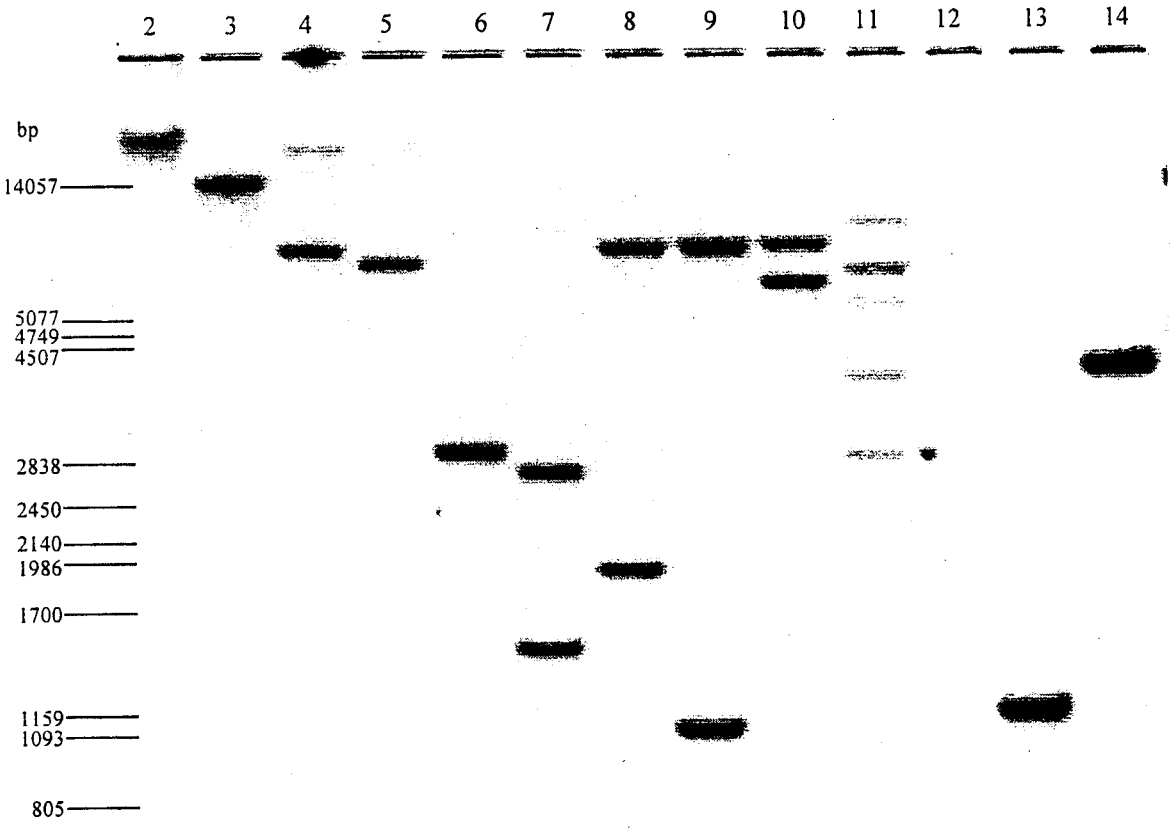
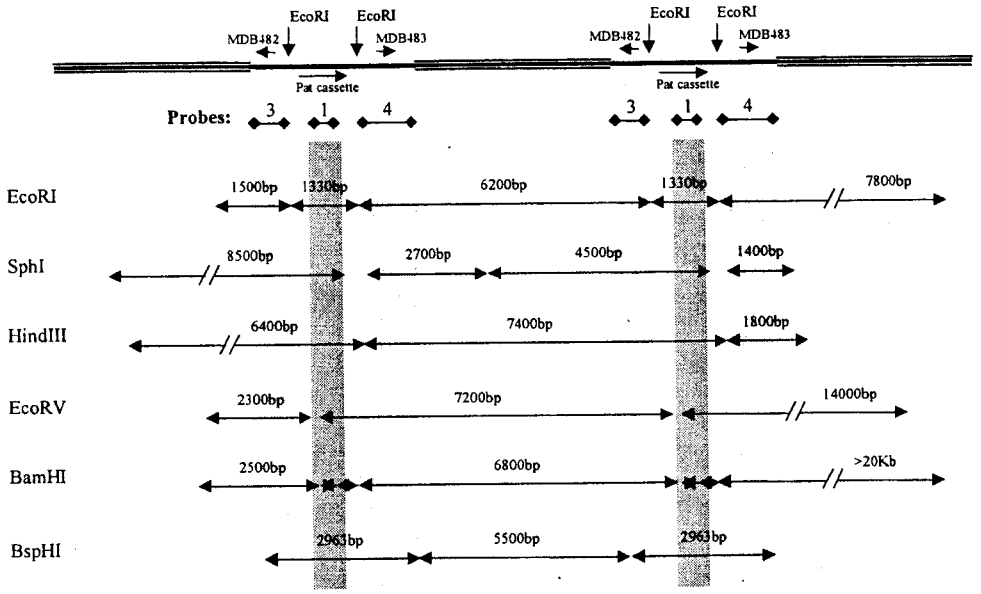


Figure 7. Schematic drawing of the insert in soybean event GU262.
Probe 1 = *pat* probe. Probe 2 = 3' *bla* probe. Probe 3 = 5' *bla* + vector probe. Probe 4 = ori + vector probe.



C. Gene Expression in Event GU262

The content of phosphinothricin acetyltransferase (PAT) protein in the transformation event GU262 was determined in leaf tissue by Enzyme Linked Immunosorbent Assay (ELISA). A polyclonal antibody was used in the ELISA . It detects both inactive and intact PAT enzyme. Therefore, the enzyme detected may not be functional.

The PAT ELISA is a sandwich immunoassay in which PAT specific antibodies are used to coat the wells and serve as capture antibodies for PAT protein. Samples consisting of transformant extracts, non-transformant extracts as controls, and pure PAT protein as a standard are added to the wells. Following incubation, during which time the PAT in the sample is captured by the coated antibodies, the unbound material is removed. The second PAT antibody is labeled with horseradish peroxidase and it binds to PAT protein captured by the first antibody. When the appropriate substrate is added the enzyme-labeled antigen-antibody complex converts it to a blue color. The resultant color development is proportional to the concentration of PAT protein in each microwell. Three dilutions of each extract are tested and the value nearest to the midpoint of the standard curve is used to determine the PAT content. ELISA assays were performed on leaf tissue from 10-day-old soybean seedlings. Results from the ELISA are shown in Table 4.

Table 4. PAT Content [a] in Leaves of GU262 as Detected by ELISA

Plant [b]	mg TEP [c] / g sample	µg PAT/ g sample	% PAT/TEP	% PAT/fresh weight (g/g)
PHI2-NT	5.1	ND [d]	0.00	0.00
GU262	4.7	3.03	0.064	3.03 x 10-4

[a] Values reported are the average from two replicate extractions from two samples of 10 day-old seedling leaves.
[b] NT = nontransformed.
[c] TEP = total extractable protein
[d] ND = not detectable. Limit of detection is 0.004 µg PAT/g matrix.

V. Agronomic Performance of Event GU262

As was seen for events A2704-12 and A5547-127, there were no differences in morphology, and in disease or insect resistance between the event GU262 and its nontransgenic counterpart. In addition, the expected segregation ratios were observed for a single dominant *pat* locus. In these trials, when sprayed with the herbicide, all plants exhibited a high level of glufosinate resistance, indicating that the gene is stably integrated and expressed.

A. Field Tests

Event GU262 was evaluated in the field in 1996 at 2 sites (Mississippi and Puerto Rico), in 1997 at 3 sites (Iowa and Mississippi), and in 1998 at 12 sites (Arkansas, Florida, Iowa, Mississippi, North Carolina) under authorizations granted by APHIS (USDA authorizations: 96-134-03N, 96-184-01N, 97-038-02N, 97-038-03N, 98-040-07N, 98-040-08N, 98-040-09N, 98-071-23N, 98-078-04N, 98-078-28N, 98-125-03N). The purpose of the trials was to increase seed, advance generations, demonstrate the agronomic performance, to evaluate segregation ratios and/or to collect samples for analytical work regarding the composition and nutrient and transgene product in transformation event GU262.

The great majority of the trials in the United States have been breeding trials, however, observations were also made on agronomic characteristics and disease and pest characteristics. The Appendix contains termination reports submitted to the USDA for the environmental releases that have been completed or as ongoing in the United States and Puerto Rico.

B. Agronomic, Disease and Pest Characteristics

Company researchers, university cooperators, and soybean breeders made visual observations of many agronomic traits of event GU262 including plant morphology, time of flowering, stand count, plant height, crop injury due to chemical application, relative maturity, lodging scores, yield, and plant standability. For all traits evaluated a nontransgenic genetic counterpart was also evaluated. Qualitative evaluations and certain quantitative evaluations were made during the 1996, 1997 and 1998 growing seasons. For all agronomic information gathered, there were no significant differences between transformation events and the nontransgenic counterparts, with the single exception that the nontransgenic material was not resistant to GA application (See termination reports in the Appendix).

Evaluation notes taken on the experiments in Puerto Rico and Mississippi in 1997 are presented in Table 5. Scores were taken for emergence, relative

maturity, plant habit, flower color, pubescence color, hila color, pod wall color at both locations. Observations for pest populations were taken at both locations. Qualitative scores for Southern root-knot nematode (*Meloidogyne javanica*) and frogeye leaf spot (*Cercospora sojina*) were noted at the Greenville, Mississippi. No differences were noted between transformation event GU262 and the non-transgenic variety PHI2.

Company researchers and cooperators made visual observations on several occasions for plant pathogenic organisms in trials containing event GU262 and its nontransgenic counterpart during the 1996, 1997 and 1998 growing seasons. Such observations revealed some minor pathogen infections but no infestations (see Appendix). Whenever pests were observed there were no differences in damage or populations found between GRS events and nontransgenic counterparts. The integration of vector DNA did not affect the inherent resistance of the parent cultivar to soybean cyst nematode. The beneficial organisms - ladybugs and earthworms - were observed in both transgenic and nontransgenic counterpart plots. As was found for the GRS events in the previously approved petition 96-068-01p and petition extension 98-014-01p, event GU262 did not influence susceptibility to disease or pest organisms in diverse genetic backgrounds and environments. In summary, the presence of the glufosinate resistant gene did not influence susceptibility to disease of pest organisms in the test environments.

VI. Potential for Environmental Impact from Noncontained Use of Event GU262

There were no significant differences, apart from the intended change to glufosinate tolerance, demonstrated in field tests of event GU262 compared with the nontransgenic parent line. No morphological, beneficial organism, disease, or pest differences between GU262 and previously considered GRS events were noted. There is no reason to think cultivation of event GU262 and its progeny will have environmental effects different from cultivation of other GRS events which have already been considered by APHIS. No adverse consequences from the introduction of GU262 are expected.

VII. Statement of Grounds Unfavorable

No unfavorable information and data has been demonstrated for GRS Transformation Event GU262.

Table 5. Agronomic Characteristics of GU262 and Nontransgenic Parent[a]

Entry	Emergence Score[b]	Relative Maturity	Plant Habit[c]	Flower Color	Pubescence Color	Hila Color	Podwall Color	Root-knot Nematode	Frogeye Leaf Spot
1	9	59	D	White	Gray	Buff	Tan	Good	Good
3	9	59	D	White	Gray	Buff	Tan	Good	Good
6	9	59	D	White	Gray	Buff	Tan	Good	Good
14	9	59	D	White	Gray	Buff	Tan	Good	Good
16	9	60	D	White	Gray	Buff	Tan	Good	Good
17	9	59	D	White	Gray	Buff	Tan	Good	Good
19	9	59	D	White	Gray	Buff	Tan	Good	Good
25	9	59	D	White	Gray	Buff	Tan	Good	Good
26	9	59	D	White	Gray	Buff	Tan	Good	Good
30	9	59	D	White	Gray	Buff	Tan	Good	Good
31	9	60	D	White	Gray	Buff	Tan	Good	Good
32	9	59	D	White	Gray	Buff	Tan	Good	Good
34	9	58	D	White	Gray	Buff	Tan	Good	Good
35	9	59	D	White	Gray	Buff	Tan	Good	Good
36	9	59	D	White	Gray	Buff	Tan	Good	Good
37	9	58	D	White	Gray	Buff	Tan	Good	Good
39	9	59	D	White	Gray	Buff	Tan	Good	Good
40	9	59	D	White	Gray	Buff	Tan	Good	Good
41	9	59	D	White	Gray	Buff	Tan	Good	Good
42	9	59	D	White	Gray	Buff	Tan	Good	Good
47	9	59	D	White	Gray	Buff	Tan	Good	Good
48	9	59	D	White	Gray	Buff	Tan	Good	Good
PHI2	9	59	D	White	Gray	Buff	Tan	Good	Good

[a] Agronomic data collected from Mississippi and Puerto Rico under authorization 97-134-03N and 96-184-01N, respectively.
[b] Emergence score: 1 = poor, 9 = excellent. [c] Plant habit: D = Determinant.

VIII. Appendix -

USDA Field Trial Termination Reports
Open Reading Frames and Associated Regulatory Regions in pB2/35SAcK
Comparison of Molecular Data for GRS Events

List of Release Authorizations

Authorization Number	States and Sites
96-134-03N	MS (1)
96-184-01N	PR (1)
97-006-02N	not planted
97-038-02N	IA (1)
97-038-03N	MS (2)
97-038-04N	not planted
98-040-07N	MS (3)
98-040-08N	AR (2)
98-040-09N	AR (1)
98-071-23N	AR (2), MS (1)
98-078-04N	IA (1)
98-078-23N	FL (1)
98-125-03N	NC (1)

Field Termination Reports

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	96-134-03N
Pioneer Number:	SOY-MS-96-03
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	January 31, 1997

PURPOSE

A breeding trial of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, hila color and pod wall color. Qualitative scores for root-knot nematode and frogeye leaf spot were noted.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered one planting site near our research station located in Greenville, Washington County, MS. The trial was planted on staggered dates and comprised 0.03 acres total:

June 13, 1996	29 rows
June 17, 1996	8 rows
June 20, 1996	12 rows
June 25, 1996	12 rows

The transformation events planted in this trial were PHI2-GU179, PHI2-GU218, PHI2-GU250, PHI2-GU262, PHI2-GU384 and PHI2-GU386. The stand of each planting was normal as expected. Plants were allowed to self-pollinate. Plants were pulled and threshed on October 18, 1996; seed was retained for future breeding efforts. The following year, the site will be left fallow.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. At three different stages (July 12, August 7 and September 9, 1996) the observations were recorded, and results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants. The site was treated with Larvin for tobacco bud worm and soybean looper, and no insect outbreaks were noted. Bean Pod Mottle Virus was the most prevalent disease occurring in the breeding nurseries, but the non-transgenic parent line is tolerant to this virus. Both the transgenic and control lines showed few (if any) symptoms.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was disked into the plot for soil composting.

POST-TRIAL MONITORING
In the 1997 growing season, the site will be left fallow and will be periodically monitored. The site will be visited multiple times in the spring of 1997 when soil temperatures reach a level at which soybean emergence will be expected. The site will be visually inspected for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. All volunteer soybean plants will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	96-184-01N
Pioneer Number:	SOY-PR-96-04
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-6883
Date Of This Report:	March 31, 1997

PURPOSE

Breeding/seed increase of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, hila color and pod wall color.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered one planting site near our research station located near Salinas, Puerto Rico. The trial was planted on staggered dates and comprised approximately 0.27 acres total.

Plant date	# rows	Harvest date
August 2, 1996	56 rows	November 20, 1996
August 21, 1996	16 rows	November 20, 1996
October 18, 1996	20 rows	February 12, 1997
October 30, 1996	102 rod rows	January 20, 1997
November 25, 1996	72 rod rows and 144 short rows	February 28, 1997
December 3, 1996	16 rows	not harvested

The transformation events planted in this trial were PHI1-GU022, PHI1-GU050, PHI1-GU057, PHI2-GU179, PHI2-GU218, PHI2-GU250, PHI2-GU262, PHI2-GU384 and PHI2-GU386. The stand of each planting was normal as expected. All grain was either destroyed by cultivating the seed into the soil below the depth of germination or else retained for future breeding efforts at the Puerto Rico station or other Pioneer research locations. The following growing cycle the site will be planted to a non-soybean crop (corn, sunflowers, plantains, bananas, etc.)

GENERAL FIELD OBSERVATIONS

Hurricane Hortense caused heavy rain (10-12") and high winds in Puerto Rico in mid-September 1996, but plants recovered satisfactorily and showed no permanent damage. The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. At several different stages (September 4, October 1, December 3 and December 30, 1996, and January 30, February 28 and March 27, 1997) the observations were recorded, and results are listed below.

There were no unanticipated morphological differences between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

Low to moderate insect pressure from soybean loopers was present; some white flies were also seen. There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was cut at ground level and plowed into the soil for composting.

POST-TRIAL MONITORING

In the post-trial growing cycle of 1997, the site will be planted to a non-soybean crop and will be periodically monitored for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. All volunteer soybean plants will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	97-006-02N
Pioneer Number:	SOY-PR-97-03N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-3499
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	August 13, 1998

Please be advised that this trial was not planted.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	97-038-02N
Pioneer Number:	SOY-IA-97-04N
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-3499
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	August 13, 1998

PURPOSE

A breeding trial of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, hila color and pod wall color. Qualitative scores for phytophthora root rot and white mold were noted at this site.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered one planting site near our research station located in Johnston, Polk County, IA. The trial was planted on May 21 and June 9, 1997 and comprised 135 rows of approximately 30 plants per row, for a total of 0.03 acres.

The transformation events planted in this trial were PHI1-GU022, PHI2-GU179, PHI2-GU210 and PHI2-GU262. The stand of each planting was normal as expected. Plants were allowed to self-pollinate. Plants were pulled and threshed in the fall of 1997; seed will retained for future breeding efforts as needed. Unused seed was destroyed. The following year, the site was rotated to corn and monitored as needed.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. The observations were recorded monthly, and results are listed below.

There were no unanticipated morphological differences (such as changes in plant architecture, heavy branching or pod shattering, switching from determinate to indeterminate, etc.) between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

A few bean leaf beetles were noted on June 30, 1997. Mild Phyphthora symptoms, affecting a few plants, were noted on August 4, 1997. There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material was disked into the plot for soil composting.

POST-TRIAL MONITORING

In the 1998 growing season, the site was rotated to corn and was periodically monitored. The site was visited multiple times in the spring of 1998. The site was visually inspected for volunteer soybean plants. The number of volunteer soybean plants was observed and recorded. All volunteer soybean plants were destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	97-038-03N
Pioneer Number:	SOY-MS-97-05
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-270-3499
Date Of This Report:	August 13, 1998

PURPOSE

A breeding trial and seed increase of glufosinate-tolerant soybeans. The following traits were recorded: flower color, crop injury due to chemical application and plant standability. Scores were taken for relative maturity, plant habit, pubescence color, hila color and pod wall color. Qualitative scores for root-knot nematode and frogeye leaf spot was noted.

SUMMARY OF EXPERIMENTAL RESULTS

This permit covered two planting sites near our research station located in Greenville, Washington County, MS. The trial was planted on staggered dates and comprised approximately 5.6 acres total:

Site	Plant dates	Purpose	Size	# plants/row
Greenville #1	June 5, 1997		44 rows	100
	June 24, 1997		144 rows	145
Greenville #2	May 22, 1997	2-row yield test	112 rows	126
	June 6, 1997		230 rows	140
	June 6, 1997	seed increase	176 rows @ 4.9 acres	130

The transformation events planted in this trial were PHI2-GU179, PHI2-GU210 and PHI2-GU262. The stand of each planting was normal as expected. Plants were allowed to self-pollinate. Plants were pulled and threshed in the fall of 1997 and seed was retained for future breeding efforts as needed. Unused seed was destroyed. The following year, the site was left fallow.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. At many different stages the observations were recorded, and results are listed below.

There were no unanticipated morphological differences (such as changes in plant architecture, heavy branching or pod shattering, switching from determinate to indeterminate, etc.) between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

No disease symptoms were noted. The following insects were noted and documented:

Greenville #1	July 7	alfalfa hoppers, grasshoppers, stink bugs, potato leaf hoppers occurring with mild symptoms
	July 16	alfalfa hoppers, with mild symptoms
	August 8	leaf hoppers, grasshoppers and a few bud worms but none producing symptoms
	August 12	grasshoppers, alfalfa hoppers, stink bugs producing very mild symptoms

Greenville #2	June 24	three-corners alfalfa hoppers, potato leafhopper, grasshoppers and bean leaf beetles noted; very mild symptoms
	July 7	leafhoppers, grasshoppers, bean leaf beetles and stink bugs noted with mild symptoms
	July 21	alfalfa hoppers, potato leaf hoppers, bean leaf beetles and bud worm noted with mild symptoms
	August 8	grasshoppers, stink bugs, bud worms and leaf hoppers noted, but no symptoms
	August 13	a few leaf hoppers, grasshoppers, alfalfa hoppers noted; some bud worms or corn ear worms producing mild symptoms

There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material was disked into the plot for soil composting.

POST-TRIAL MONITORING
In the 1997 growing season, the site was left fallow and was periodically monitored. The site was visited multiple times in the spring of 1997 when soil temperatures reach a level at which soybean emergence was expected. The site was visually inspected for volunteer soybean plants. The number of volunteer soybean plants was observed and recorded. All volunteer soybean plants were destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate.

SUMMARY REPORT OF FIELD TEST DATA

Approved Permit Number:	97-038-04N
Pioneer Number:	SOY-AR-97-06
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Tracy Rood 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-4036
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	August 14, 1997

Please be advised that this trial was not planted.

INTERIM REPORT OF FIELD TEST DATA

Approved Permit Number:	98-040-07N
Pioneer Number:	SOY-US-MS-98-13
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-3499
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	July 23, 1998

PURPOSE

A breeding trial of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, and will be taken for hila color and pod wall color. Qualitative scores for phytophthora root rot and white mold were noted at this site.

SUMMARY OF EXPERIMENTAL RESULTS

The permit covered three planting sites: Cary, Sharkey County, MS; Greenville, Washington County, MS; and Clarksdale, Coahoma County, MS.

Site	Planting Date	Number of Rows	Number of Plants per row
Cary, Sharkey County	May 6, 1998	48	150
Greenville, Washington County	May 4, 1998	728	150
Clarksdale, Coahoma County	May 4, 1998	48	150

The transformation event planted in these trials was PHI2-GU262. The stand of each planting was normal as expected. Plants were allowed to self-pollinate. Plants will be pulled and threshed in the fall of 1998; seed will be retained for future breeding efforts as needed. Unused seed will be destroyed. The following year, the site will be rotated to corn and monitored as needed.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. The observations were recorded monthly, and results are listed below.

There were no unanticipated morphological differences (such as changes in plant architecture, heavy branching or pod shattering, switching from determinate to indeterminate, etc.) between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

Mild levels of grasshoppers and bud worms were observed on June 6, 1998 at the Cary, Sharkey County, MS site. At all of the trials, there were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION
All remaining vegetative material will be disked into the plot for soil composting.

POST-TRIAL MONITORING
In the 1999 growing season, the site will be rotated to corn and will be periodically monitored. The site will be visited multiple times in the spring of 1999 when soil temperatures reach a level at which soybean emergence will be expected. The site will be visually inspected for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. All volunteer soybean plants will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

INTERIM REPORT OF FIELD TEST DATA

Approved Permit Number:	98-040-08N
Pioneer Number:	SOY-US-AR-98-14
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-3499
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	July 23, 1998

PURPOSE

A breeding trial of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, and will be taken for hila color and pod wall color. Qualitative scores for phytophthora root rot and white mold were noted at this site.

SUMMARY OF EXPERIMENTAL RESULTS

The permit covered two planting sites: Wilson, Mississippi County, AR and Stuggart, Arkansas County, AR

Site	Planting Date	Number of Rows	Number of Plants per row
Wilson, Mississippi County	May 14, 1998	208	150
Stuggart, Arkansas County	May 14, 1998	48	150

The transformation event planted in this trial was PHI2-GU262. The stand of each planting was normal as expected. Plants were allowed to self-pollinate. Plants will be pulled and threshed in the fall of 1998; seed will be retained for future breeding efforts as needed. Unused seed will be destroyed. The following year, the site will be rotated to corn and monitored as needed.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. The observations were recorded monthly, and results are listed below.

There were no unanticipated morphological differences (such as changes in plant architecture, heavy branching or pod shattering, switching from determinate to indeterminate, etc.) between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

Bean leaf beetles are a common pest in the immediate growing area. Monthly field observations reported no unusual bean leaf beetle activity. There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material will be disked into the plot for soil composting.

POST-TRIAL MONITORING

In the 1999 growing season, the site will be rotated to corn and will be periodically monitored. The site will be visited multiple times in the spring of 1999 when soil temperatures reach a level at which soybean emergence will be expected. The site will be visually inspected for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. All volunteer soybean plants will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

INTERIM REPORT OF FIELD TEST DATA

Approved Permit Number:	98-040-09N
Pioneer Number:	SOY-US-AR-98-24
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-3499
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	July 23, 1998

PURPOSE

A breeding trial of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, and will be taken for hila color and pod wall color. Qualitative scores for phytophthora root rot and white mold were noted at this site.

SUMMARY OF EXPERIMENTAL RESULTS

The trial was planted at Delaplaine, Greene County, Arkansas. The trial was planted on June 1, 1998 and comprised of approximately 65 acres.

The transformation event planted in this trial was PHI2-GU262. The stand was normal as expected. Plants were allowed to self-pollinate. Plants will be pulled and threshed in the fall of 1998; seed will be retained for future breeding efforts as needed. Unused seed will be destroyed. The following year, the site will be rotated to corn and monitored as needed.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. The observations were recorded monthly, and results are listed below.

There were no unanticipated morphological differences (such as changes in plant architecture, heavy branching or pod shattering, switching from determinate to indeterminate, etc.) between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

A few bean leaf beetles were noted on June 30, 1998. There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material will be disked into the plot for soil composting.

POST-TRIAL MONITORING

In the 1999 growing season, the site will be rotated to corn and will be periodically monitored. The site will be visited multiple times in the spring of 1999 when soil temperatures reach a level at which soybean emergence will be expected. The site will be visually inspected for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. All volunteer soybean plants will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

INTERIM REPORT OF FIELD TEST DATA

Approved Permit Number:	98-078-04N
Pioneer Number:	SOY-US-IA-98-12
Name:	Pioneer Hi-Bred International, Inc.
Institute Address:	c/o Jeffrey Rowe 7100 NW 62nd Ave. PO Box 1000 Johnston, IA 50131-1000
Telephone Number:	515-270-3499
Facsimile Telephone Number:	515-334-4478
Date Of This Report:	July 23, 1998

PURPOSE

A breeding trial of glufosinate-tolerant soybeans. The following traits were recorded: plant morphology, time to flowering, stand count, plant height, crop injury due to chemical application and plant standability. Scores were taken for emergence, relative maturity, plant habit, flower color, pubescence color, and will be taken for hila color and pod wall color. Qualitative scores for phytophthora root rot and white mold were noted at this site.

SUMMARY OF EXPERIMENTAL RESULTS

The trial was planted at our research site in Johnston, Polk County, Iowa. Planting occurred on May 28, 1998 and comprised of 834 transgenic rows with approximately 35 plants per row.

The transformation event planted in these trials was PHI2-GU262. The stand was normal as expected. Plants were allowed to self-pollinate. Plants will be pulled and threshed in the fall of 1998; seed will be retained for future breeding efforts as needed. Unused seed will be destroyed. The following year, the site will be rotated to corn and monitored as needed.

GENERAL FIELD OBSERVATIONS

The plants were frequently observed by personnel experienced in soybean breeding and plant pathology during the growing season. The observations were recorded monthly, and results are listed below.

There were no unanticipated morphological differences (such as changes in plant architecture, heavy branching or pod shattering, switching from determinate to indeterminate, etc.) between the transformed organisms and appropriate unmodified controls.

Observations of the modified plants showed fertility and seed set comparable with that of the nonmodified controls.

Observations of the modified plants did not reveal novel characteristics associated with weediness.

Observations of flowering characteristics did not disclose any deviations from the parental/control outcrossing potential.

Mild levels of bean leaf beetles were observed on June 15, 1998 and July 15, 1998. There were no apparent differences in disease or insect susceptibility of transgenic vs. non-transgenic plants.

Observations did not disclose characteristics of the modified organisms that would increase the long-term survival of any progeny that might have escaped the test area.

There were no indications of potential adverse human health effects or impacts on the health of people living in the area of the test.

FINAL DISPOSITION

All remaining vegetative material will be disked into the plot for soil composting.

POST-TRIAL MONITORING

In the 1999 growing season, the site will be rotated to corn and will be periodically monitored. The site will be visited multiple times in the spring of 1999 when soil temperatures reach a level at which soybean emergence will be expected. The site will be visually inspected for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. All volunteer soybean plants will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than glufosinate. If any volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

Interim Report of Field Test Data for Glufosinate Resistant Soybean Transformation Event GU262 AgrEvo USA Company

Notification Numbers: 98-071-23N, 98-078-28N, 98-125-03N

Number of States and Sites: AR (2), FL (1), MS (1), NC (1)

Planting Dates: June 1-June 11, 1998

Purpose:

Trials were conducted to collect samples for analyitcal work regarding the composition and nutritional of the soybeans and content of the transgene product in them. In addition, the following items were recorded: percent emergence, stand count, disease and insect susceptibility, presence of beneficial organisms, and crop injury due to chemical application.

The transformation event planted in these trials was GU262, also referred to as PHI2-GU262. The nontransgenic parent, PHI2, was planted for comparison.

General Field Observations:

The plots were observed by personnel with experience in soybean cultivation. Recorded observations of obvious differences were provided for approximately the V3 (end of June) and V8 (mid-end of July) stages of soybean growth.

Emergence was recorded at approximately the V3 stage and ranged from 28-97% for GU262 and 70-95% for the nontransgenic counterpart. Stand count ranged from 2.9-8.3 plants per foot for GU262 and 5.5-10 plants per foot for the nontransgenic counterpart. The stand count was generally a reflection of the seeding rate at each site such that the stand count was less when the seeding rate was lower. At one site in Arkansas the germination rate was low for both the transgenic and nontransgenic although the seeding rate was in the mid-range. This is attributed to the seed having been planted deeper than usual because of the dryness of the soil at planting time.

The following disease and insect pests were specifically scouted for – brown spot, soybean cyst nematode, downy mildew, Phytophthora root rot, army worm, corn earworm and green stinkbug. None were observed in either the transgenic or nontransgenic plots. Rhizoctonia root rot was observed in less than 1% of the plants, both transgenic and nontransgenic, at the V1 stage for the Florida site. At one of the Arkansas sites soybean looper and 3-colored alfalfa hopper slight feeding activity was equally observed on both the transgenic and nontransgenic plants at the V8-V9 stage. No other disease or insect pests were noted.

The beneficial organisms - ladybugs and earthworms - were specifically scouted for and no differences were noted between the GU262 and nontransgenic plots with regard to these organisms. No other beneficial organisms were observed.

At sites where Liberty® Herbicide (glufosinate-ammonium) was applied to the transgenic plots no crop injury was observed with the exception that one plant in one GU262 plot was evidently killed by the herbicide. Since GU262 breeding is not complete it is probable that some Liberty susceptible plants are present in the population. There were more weeds present in untreated plots than in treated plots of GU262, as would be expected.

Both GU262 and nontransgenic counterparts appeared similar in development.

Final Disposition:

At the North Carolina site the crop was tilled into the soil on 21 July, as the test was complete. Following the final collection of samples from the other sites all the remaining vegetative material will be disked into the plot for soil composting.

Post-Trial Monitoring:

In the 1999-growing season the sites will be visually inspected for volunteer soybean plants. The number of volunteer soybean plants will be observed and recorded. Any volunteers observed will be destroyed by mechanical means, removed by hand weeding, or destroyed with herbicides other than Liberty. If volunteers are observed, the numbers and action taken will be reported to APHIS at that time.

Open Reading Frames and Associated Regulatory Regions in pB2/35SAcK

Vector pB2/35SAcK contains two open reading frames, *bla* and *pat*. Only the *pat* reading frame is functional and intact in the event GU262. The event is considered a regulated article because it contains DNA sequences from CaMV and *A. tumefaciens*. This section contains a more thorough description of the inserted genetic material responsible for expression of the glufosinate resistance trait. The *bla* gene is also addressed. Refer to "Genetic Elements" table for a description of all other introduced genetic sequences in pB2/35SAcK.

1. CaMV *35S* promoter and terminator The *35S* promoter and terminator sequences are derived from CaMV (Odell et al., 1985). The promoter controls transcription initiation of the *pat* gene. The terminator ends transcription of the *pat* gene. CaMV is a doublestranded DNA caulimovirus with a host range restricted primarily to cruciferous plants. The *35S* promoter directs high level constitutive expression and is widely used as a promoter for high expression of genes (Harpster et al., 1988). The CaMV sequences, as used in the GRS, do not cause the soybean to become a plant pest.

2. *pat* The *pat* gene is a synthetic version of the *pat* gene isolated from *S. viridochromogenes*, strain Tü 494 (Bayer et al., 1972). Since the native *pat* gene has a high G:C content, which is atypical for plants, a modified nucleotide sequence was synthesized using codons preferred by plants. The amino acid sequence of the enzyme remains unchanged. The *pat* genes encode the enzyme phosphinothricin acetyltransferase (PAT), which imparts resistance to the phytotoxic activity of GA.

Members of the genus *Streptomyces* are gram-positive sporulating soil bacteria. These organisms synthesize numerous unique compounds, secondary metabolites, that often possess antibacterial, antitumor, or antiparasitic activity (Demain et al., 1983). One such compound, the antibiotic bialaphos, is produced by both *S. viridochromogenes* and *S. hygroscopicus*. Bialaphos (syn. L-phosphinothricyl-L-alany-L-alanine) is an herbicidally active tripeptide consisting of two L-alanine molecules and an analog of L-glutamic acid called phosphinothricin. When it is released by peptidases, the L-phosphinothricin moiety , is a potent inhibitor of glutamine synthetase (GS) (Bayer at el. 1972). L-phosphinothricin is the active component of the commercial herbicides, Herbiace ® (Meiji Seika Ltd.) and Basta, Ignite®, Rely®, Liberty ® and Harvest® and Finale® (AgrEvo GmbH). Herbiace® is bialaphos that is commercially produced using *S. hygroscopicus*. The other herbicides are the ammonium salts of phosphinothricin, common name GA, and are chemically synthesized.

L-phosphinothricin is a potent inhibitor of the enzyme GS in both bacteria and plants, where it apparently binds competitively to the enzyme by displacing L-

glutamate from the active site. Evidently GS binds L-phosphinothricin better than the substrate. GS plays a central role in nitrogen metabolism of higher plants where it is the only enzyme in plants that can detoxify ammonia released by nitrate reduction, amino acid degradation and photorespiration (Miflin and Lea, 1976). Ammonia, although a plant nutrient and metabolite, is toxic in excess and leads to death of plant cells (Tachibana et al., 1986).

Although the GS from both *S. viridochromogenes* and *S. hygroscopicus* are sensitive to L-phosphinothricin, the bacteria produce an inactivating enzyme, PAT. PAT catalyzes the conversion of L-phosphinothricin to N-acetyl-L-phosphinothricin in the presence of acetyl CoA as a co-substrate. N-acetyl-L-phosphinothricin does not inactivate GS, and, thus, has no herbicidal activity. Therefore, plants expressing the PAT enzyme are resistant to the phosphinothricin class of herbicides. The PAT enzyme is encoded by the *bar* (*b*ialaphos-*r*esistance) gene in *S. hygroscopicus*, and by the *pat* gene in *S. viridochromogenes*. These genes function both as an integral part of the biosynthetic pathway of bialaphos and as an enzyme which confers resistance (Kumada, 1986).

3. *bla* The ß-lactamase gene was isolated from pBR322, a plasmid of *E. coli* (Sutcliffe, 1978). It encodes a β-lactamase. β-lactamase genes are found throughout nature (Sykes and Smith, 1979) . The gene is expressed in bacteria where it is used in the selection of transformed bacteria, which are then used to amplify the plasmid vector.

Genetic Elements of the Vector pB2/35SAcK

Genetic element	Position in vector	Size (Kb)	Function
RB	189-243	0.054	Right border sequence of *A. tumefaciens* Ti plasmid pTiAch5 (Gielen et al., 1984).
P-35S	461-1003	0.54	The CaMV promoter of the *35S* transcript. (Odell et al., 1985)
pat	1012-1563	0.55	The synthetic glufosinate resistance gene. (Eckes et al., 1989)
T-35S	1582-1784	0.20	The CaMV 3´-nontranslated region of the *35S* transcript (Pietrzak et al., 1986).
ori-pUC	2253-2803	0.55	Origin of replication (ColE1) of pUC18. (Yanisch-Perron et al., 1985)
bla	3876-3016	0.86	Ampicillin resistance gene from *E. coli* expresses a ß-lactamase only in bacteria (Sutcliffe, 1978).

Literature Cited

Bayer, E., Gugel, K.H., Hagele, K., Hagenmaier, H., Jessipow, S., König, W.A., Zähner, H. (1972) Stofwechproduckte von Mikroorganismen. Phosphinothricin und Phosphinothricyl-alanyl-alanin. Helvetica Chimica Acta 55: 224-239.

Demain, A.L., Aharonowitz, Y., Martin, J.-F. (1983) Metabolic control of secondary biosynthetic pathways. *In:* Biochemistry and genetic regulation of commercially important antibiotics, Vining, L.C. (ed.). Addison-Wesley Publishing Co., Reading, Massachusetts. pp. 49-72.

Harpster, M.H., Townsend, J.A., Jones, J.D.J., Bedbrook, J., Dunsmuir, P. (1988) Relative strengths of the 35S cauliflower mosaic virus, 1', 2' and nopaline synthase promoters in transformed tobacco, sugarbeet and oilseed rape callus tissue. Molecular and General Genetics 212: 182-190.

Kumada, Y., Anzai, H., Takano, E., Murakami, T., Hara, O., Itoh, R., Imai, S., Satoh, A., Nagaoka, K. (1988) The bialaphos resistance gene (*bar*) plays a role in both self-defense and bialaphos biosynthesis in *Streptomyces hygroscopicus*. Journal of Antibiotics 41: 1838-1845.

Miflin, B.J, and Lea, P.J. (1976) The pathway of nitrogen assimilation in plants. Phytochemistry 15: 873-885.

Odell, J.T., nagy, F., Chua, N.-H. (1985) Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. Nature 313: 810-812.

Sykes, R.B., and Smith, J.T. (1979) Biochemical aspects of β-lactamases from gram-negative organisms. *In*: Beta-lactamases, Hamilton-Miller, J.M.Y. and Smith, J.T. (eds.). Academic Press, New York. pp. 369-401.

Tachibana, K., Watanabe, T., Sekizuwa, Y., Takematsu, T. (1986) Accumulation of ammonia in plants treated with bialaphos. Journal of Pesticide Science 11: 33-37.

Comparison of Molecular Data [1] for GRS Events: A2704-12, A2704-21, A5547-35, A5547-127 and GU262

Event	*pat* probe – # bands detected **HindIII digest**	*bla* probe – # bands detected **DraI digest**
A2704-12	4 [2]	2 [1, 5]
A2704-21	5 [2]	3 [1, 5]
A5547-35	1 [2]	0 [1, 5]
A5547-127	1 [3], 1 intact	2 [3, 5], 1- 3', 1-5'
GU262	2 [4], 2 intact	2 [4], 0- 3', 2- 5'

(1) Data shown for shared GRS DNA event digests.
(2) From Southerns in petition # 96-068-01p.
(3) From Southerns in petition extension # 98-014-01p.
(4) From Southern data presented in this extension.
(5) No intact *bla* genes detected.

AgrEvo

98-238-01p

September 30, 1998

Ms. Rebecca Bech
Director, Scientific Services
USDA, APHIS, PPQ, SS
4700 River Road, Unit 147
Riverdale, MD 20737-1237

Re: **Supplemental Information for 'Extension of a Determination of Nonregulated Status for Glufosinate Resistant Soybean Transformation Events (96-068-1p)' – 98-238-01p**

Dear Ms. Bech:

AgrEvo USA Company is submitting supplemental information for "Extension of the Determination of Nonregulated Status to the Animal and Plant Health Inspection Service (APHIS) for Glufosinate Resistant Soybean (GRS) Events (96-0681p) confirming that the 5' *bla* fragments found in event GU262 are not expressed. No expression of the *bla* sequences is expected since this gene is under the control of bacterial expression signals and should only be expressed in bacteria. Nevertheless, cryptic gene expression in this event was analyzed by Northern analysis of RNA extracted from several tissues and hybridized with a single stranded RNA probe homologous to the *bla* gene.

In the positive control lanes (Figure 1s, lanes 10-17) an approximately 1000 nucleotide transcript is expected to hybridize with the probe. This is the size of the *in vitro* synthesized sense RNA. Hybridization was observed in lanes 13-17 of the dilution series. The limit of detection for this experiment is 2 pg (Figure 1s, lane 13). These results demonstrate that the Northern analysis was performed under conditions allowing hybridization to the target sequences. No positive signal could be detected in any of the tested tissues (root, stem, leaf) from GU262 and negative controls (PHI2) (Figure 1s, lanes 3-8).

Enclosed are two copies of this supplemental information. The enclosed does not contain any confidential business information.

AgrEvo USA Company
Little Falls Centre One, 2711 Centerville Road, Wilmington, DE 19808, Telephone: (302) 892-3000, Fax: (302) 892-3013

10/1/98
Kay

Please contact me at (302) 892-3155 if you have any questions concerning our petition.

Best Regards,

Sally Van Wert

Sally Van Wert, Ph.D.
Manager, Regulatory Affairs - Biotechnology

Enclosures (2)

Figure 1s. Northern Blot Analysis: Soybean Event GU262- *bla* probe. Lanes 3-8. RNA (5 μg) extracted from several tissues from GRS event GU262 and the nontransgenic parent line, PHI2. Lanes 10-17. *in vitro* synthesized sense *bla* RNA (0.1 - 32pg) and PHI2 leaf RNA (5 μg). A single stranded RNA probe (approximately 1000 nt) homologous to the *bla* gene was used as probe. Lane 3. GU262 leaf RNA. Lane 4. PHI2 leaf RNA. Lane 5. GU262 stem RNA. Lane 6. PHI2 stem RNA. Lane 7. GU262 root RNA. Lane 8. PHI2 root RNA. Lane 9. Blank . Lane 10. 0.1pg sense *bla* RNA. Lane 11. 0.5pg sense *bla* RNA. Lane 12. 1pg sense *bla* RNA. Lane 13. 2pg sense *bla* RNA. Lane 14. 4pg sense *bla* RNA. Lane 15. 8pg sense *bla* RNA. Lane 16. 16pg sense *bla* RNA. Lane 17. 32pg sense *bla* RNA. RNA MW marker (G319, Promega Corporation) sizes given in nucelotides.