Appendix 2

Determination of the 5' End of the *Arabidopsis thaliana* SEC61γ Subunit Transcript in Cultivance Soybean Event 127

DETERMINATION OF THE 5' END OF THE *ARABIDOPSIS THALIANA SEC61γ* SUBUNIT TRANSCRIPT IN CULTIVANCE SOYBEAN EVENT 127

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STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA §10(d) (1) (A), (B), or (C).

Company: BASF Plant Science, L.L.C.

Company Agent: [redacted] Date: 26 Feb 2007

Title: Regulatory Affairs Manager

Signature: [redacted]

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STATEMENT OF COMPLIANCE

This study was not conducted in compliance with the requirements of 40 CFR Part 160.

The data generated by BASF Plant Science in support of product safety comply with generally accepted scientific procedures. Record keeping is consistent with procedures used throughout the research community. This report accurately presents the raw data developed during the study.

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ABBREVIATIONS AND DEFINITIONS

~	approximately
ahasl	imidazolinone-tolerant acetohydroxyacid synthase large subunit
AHASL	acetohydroxyacid synthase large subunit
bp	base pairs
cDNA	complementary DNA
CIP	calf intestinal phosphatase
Col-0	*Arabidopsis thaliana* Columbia-0 ecotype
csr1-2	*Arabidopsis thaliana* acetohydroxyacid synthase large subunit gene containing a single mutation (Ser653Asn) which confers resistance to imidazolinone herbicides
DNA	deoxyribonucleic acid
DNase I	deoxyribonuclease I
F8	eighth filial generation
gDNA	genomic DNA
mRNA	messenger RNA
PCR	polymerase chain reaction
RLM-5'-RACE	RNA-ligase mediated rapid amplification of 5' complementary DNA ends
RNA	ribonucleic acid
RNase	ribonuclease
RT-PCR	reverse transcription-polymerase chain reaction
Ser653Asn	serine residue at position 653 of *Arabidopsis thaliana* acetohydroxyacid synthase large subunit replaced with asparagine
TAP	tobacco acid pyrophosphatase
UTR	untranslated region

DETERMINATION OF THE 5' END OF THE *ARABIDOPSIS THALIANA SEC61γ* SUBUNIT TRANSCRIPT IN CULTIVANCE SOYBEAN EVENT 127

SUMMARY

Cultivance Soybean Event 127 was produced by the introduction of an imidazolinone-tolerant acetohydroxyacid synthase large subunit (*ahasl*) gene from *Arabidopsis thaliana* into the soybean plant genome, resulting in soybean plants that are resistant to imidazolinone herbicides. The majority of the Arabidopsis *SEC61γ* subunit gene, including the entire Arabidopsis *SEC61γ* coding sequence, was also introduced into the soybean genome in Cultivance Soybean Event 127. This Arabidopsis *SEC61γ* subunit gene has been shown by reverse transcription-polymerase chain reaction (RT-PCR) experiments to be weakly transcribed in Cultivance Soybean Event 127 leaves (Shen, 2007). In this study, the 5' end of the Arabidopsis *SEC61γ* subunit transcript in Cultivance Soybean Event 127 was determined by RNA-ligase mediated rapid amplification of 5' complementary DNA ends (RLM-5'-RACE). The Arabidopsis *SEC61γ* subunit transcript in Event 127 is actually a transcriptional fusion with a short segment (89 nucleotides) of adjacent soybean flanking sequence in the longest RLM-5'-RACE clone identified in this study. However, the first "ATG" codon in the longest RLM-5'-RACE cDNA sequence coincides with the predicted translation initiation site of the Arabidopsis *SEC61γ* subunit. As there are no changes at the nucleotide level, the predicted protein sequence of the Arabidopsis *SEC61γ* subunit in Cultivance Soybean Event 127 does not differ from that in Arabidopsis.

INTRODUCTION

Soybean (*Glycine max* L.) plants have been developed that are tolerant to the imidazolinone class of agricultural herbicides. The herbicide-tolerant soybean plants, referred to as Cultivance Soybean Event 127, were produced by introduction of an imidazolinone-tolerant acetohydroxyacid synthase large subunit (*ahasl*) gene from *Arabidopsis thaliana* into the soybean plant genome via biolistics using the PvuII fragment of transformation vector pAC321 (Figure 1). This fragment includes what, at the time of transformation, was defined as the Arabidopsis *AHASL* promoter, the herbicide-tolerant Arabidopsis *ahasl* coding sequence, and the Arabidopsis *AHASL* terminator. Also included in the region originally annotated as the *AHASL* promoter was the majority of the Arabidopsis *SEC61γ* subunit gene (Arabidopsis Genome Initiative locus code At3g48570), including the entire Arabidopsis *SEC61γ* coding sequence. This Arabidopsis *SEC61γ* subunit gene has since been shown by reverse transcription-polymerase chain reaction (RT-PCR) experiments to be weakly transcribed in Cultivance Soybean Event 127 leaves (Shen, 2007). It was not obvious what promoter is driving this transcription as only 18 base pairs (bp) of the putative Arabidopsis *SEC61γ* native promoter were transferred into the soybean genome in Cultivance Soybean Event 127

(Shen, 2007). Therefore, the purpose of this study was to determine the 5' end of the Arabidopsis *SEC61γ* subunit transcript in Cultivance Soybean Event 127 and to deduce the polypeptide sequence encoded by this transcript.

MATERIALS AND METHODS

Test material. The test material for this study was RNA isolated from greenhouse-grown Cultivance Soybean Event 127 plant material.

Control material. The control material for this study was RNA isolated from greenhouse-grown conventional soybean variety Conquista plant material.

Reference material. The reference material for this study was RNA isolated from *Arabidopsis thaliana* ecotype Columbia (Col-0).

Source of plant materials. Leaf tissue from the Cultivance Soybean Event 127 eighth filial (F8) generation and its comparator non-transgenic line, Conquista, was obtained from young (25-day-old) greenhouse-grown plants. Plant tissue from greenhouse-grown Arabidopsis was also used.

RNA isolation and quantitation methods. Messenger RNA (mRNA) was isolated from Arabidopsis and soybean leaf tissue using the FastTrack MAG Maxi mRNA Isolation Kit (Invitrogen GmbH; Karlsruhe, Germany). Approximately 200 mg of leaf tissue was ground in liquid nitrogen using a mortar and pestle. Pre-mixed Lysis Buffer L4/Protein Degrader (510 µl) was added to each sample and samples were suspended by vortexing. Samples were centrifuged for five minutes at approximately 16,100 x g at room temperature. Supernatants were transferred to new tubes and incubated for 15 minutes at 45˚C. FastTrack MAG Beads (200 µl per sample) were pre-washed and bound to each sample in accordance with the manufacturer's instructions. The recommended wash procedure was followed with the inclusion of an optional DNase I digestion. Two additional washes (beyond the standard protocol) with Wash Buffer W7 were performed immediately before elution of the mRNA. Messenger RNA was eluted under the recommended conditions in 20 µl RNase-free water followed by a second elution in 10 µl RNase-free water. The two mRNA eluates were combined and stored at –80˚C.

Messenger RNA was diluted in 1 M Tris-HCl, pH 7.5 and quantitated by measuring the absorbance at 260 nm using a BioPhotometer (Eppendorf AG; Hamburg, Germany).

RNA-ligase mediated rapid amplification of 5' complementary DNA (cDNA) ends. RNA-ligase mediated rapid amplification of 5' cDNA ends (RLM-5'-RACE) was conducted using the GeneRacer Kit (Invitrogen) in accordance with the manufacturer's instructions unless otherwise noted. Messenger RNA (250 ng) was dephosphorylated with calf intestinal phosphatase (CIP) to remove the 5' phosphate from any truncated mRNA or non-mRNA present in the mRNA preparation. Because CIP has no effect on capped full-length mRNA, this dephosphorylation step was expected to eliminate, or at

least reduce, the contribution from non-full-length messages in later steps of the RLM-5'-RACE procedure.

This dephosphorylated RNA was then treated with tobacco acid pyrophosphatase (TAP) to remove the 5' cap structure from full-length mRNA. In order to determine the contribution from non-full-length messages in this RLM-5'-RACE experiment, a second set of samples was processed identically to the experimental samples with the exception that TAP was excluded from these control reactions. If the RLM-5'-RACE experiment is only amplifying capped full-length message, then no PCR products would be evident in these "minus TAP" controls.

TAP treatment generates a free 5' phosphate which is required for the subsequent step: ligation of the GeneRacer RNA Oligo to the 5' end of the de-capped full-length mRNA with T4 RNA ligase. The ligated mRNA was reverse transcribed into first-strand cDNA using SuperScript III RT (Invitrogen) and the GeneRacer Oligo dT Primer in a 75 minute reaction. The region of the first-strand cDNA corresponding to the GeneRacer RNA Oligo contains binding sites for the GeneRacer 5' Primer and the GeneRacer 5' Nested Primer which will be used in the primary and secondary PCR amplifications, respectively.

Primary PCR amplification was performed using 2 µl of each reverse transcription reaction as a template for amplification by Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen) under the reaction conditions specified in the GeneRacer Kit instructions. In this primary PCR, the GeneRacer 5' Primer was paired with either gene-specific primer RS41 (Integrated DNA Technologies; Coralville, Iowa) or gene-specific primer RS46 (BASF AG Oligonucleotide Synthesis Facility; Ludwigshafen, Germany). Gene-specific primer RS41, when used in combination with the GeneRacer 5' Primer, was expected to amplify the 5' end of cDNAs derived from endogenous soybean *Sec61γ* subunit transcripts. Due to the lack of a published full-length cDNA sequence, soybean *Sec61γ* subunit-specific primers were designed based on sequences from The Institute for Genomic Research's *Glycine max* gene index of likely genes and their variants. Gene-specific primer RS46, when used in combination with the GeneRacer 5' Primer, was expected to amplify the 5' end of cDNAs derived from Arabidopsis *SEC61γ* subunit (locus At3g48570) transcripts. Although there are two additional putative *SEC61γ* subunit genes in Arabidopsis, primer RS46 was designed to bind specifically within the 3' untranslated region (UTR) of only the locus At3g48570 cDNA to reduce background amplification in control RLM-5'-RACE experiments using Arabidopsis mRNA.

Gene-Specific Primers Used in RLM-5'-RACE Experiment: Primary PCR

Name	Primer Sequence	Description
RS41	CATCCTAACCAGATCCGACGATGATGTTG	*Sec61γ* subunit, soybean. Reverse primer.
RS46	CAAAGGGCTGATAATGTCGTTTGGTTCGTTCTTC	*SEC61γ* subunit, Arabidopsis. Reverse primer.

Primary PCR amplification was carried out on a PrimusHT thermal cycler (MWG-Biotech AG; Ebersberg, Germany) using the following thermal cycling conditions:

Primary PCR Thermal Cycling Parameters

Temperature	Time	Number of Cycles
94˚C	2 minutes	1
94˚C 72˚C	30 seconds 2 minutes	5
94˚C 70˚C	30 seconds 2 minutes	5
94˚C 60˚C 68˚C	30 seconds 30 seconds 2 minutes	25
68˚C	10 minutes	1
8˚C	∞	

In order to increase both yield and specificity, a secondary PCR amplification was performed using nested primers. The secondary PCR utilized 1 µl of each primary amplification reaction as a template for amplification in 50 µl reactions containing final concentrations (or quantities) of 2.5 units of Platinum *Taq* DNA Polymerase High Fidelity (Invitrogen), 1X High Fidelity PCR Buffer, 2 mM magnesium sulfate, 0.2 µM of each primer, and 0.2 mM of each dNTP (Invitrogen). In this secondary PCR, the GeneRacer 5' Nested Primer was paired with either nested gene-specific primer RS43 (Integrated DNA Technologies) or nested gene-specific primer RS42 (Integrated DNA Technologies). Nested gene-specific primer RS43, when used in combination with the GeneRacer 5' Nested Primer, was expected to amplify the 5' end of cDNAs derived from endogenous soybean *Sec61γ* subunit transcripts. Nested gene-specific primer RS42, when used in combination with the GeneRacer 5' Nested Primer, was expected to amplify the 5' end of cDNAs derived from Arabidopsis *SEC61γ* subunit (locus At3g48570) transcripts. Although there are two additional putative *SEC61γ* subunit genes in Arabidopsis, primer RS42 was designed to bind specifically within the 3' untranslated region (UTR) of only the locus At3g48570 cDNA to reduce background amplification in control RLM-5'-RACE experiments using Arabidopsis mRNA.

Gene-Specific Primers Used in RLM-5'-RACE Experiment: Secondary PCR

Name	Primer Sequence	Description
RS42	CCTACTCCTCGGAGCATTGCCTCGTA	*SEC61γ* subunit, Arabidopsis. Reverse primer, nested. Spans intron.
RS43	CAGTACGGACGGCAACCTTGGAGAAT	*Sec61γ* subunit, soybean. Reverse primer, nested.

Secondary PCR amplification was carried out on a PrimusHT thermal cycler (MWG-Biotech AG) using the following thermal cycling conditions:

Secondary PCR Thermal Cycling Parameters

Temperature	Time	Number of Cycles
94˚C	2 minutes	1
94˚C 65˚C 68˚C	30 seconds 30 seconds 2 minutes	20
68˚C	10 minutes	1
8˚C	∞	

Secondary PCR products (20 µl) were separated by electrophoresis at 10 V/cm in a 1% (w/v) agarose gel containing 0.2 µg/ml ethidium bromide in TAE (40 mM Tris-acetate, 1 mM EDTA).

Cloning of RLM-5'-RACE PCR products. The Cultivance Soybean Event 127 RLM-5'-RACE secondary PCR products generated with Arabidopsis *SEC61γ* subunit-specific primers were cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen). TOPO vector pCR4-TOPO (1 µl) was incubated with an aliquot of the secondary PCR (4 µl) and 1 µl of a 1.2 M sodium chloride, 0.06 M magnesium chloride solution for 30 minutes at 23˚C. An aliquot of the cloning reaction (2 µl) was transformed into One Shot TOP10 Chemically Competent *Escherichia coli* (Invitrogen) according to manufacturer's instructions and plated on Luria-Bertani agar plates containing 50 µg/ml kanamycin.

Twelve transformants were screened for positive clones by colony PCR. Each colony was suspended in 4 µl of sterile water. One microliter of this suspension was added to a 25 µl reaction containing final concentrations of 1X Eppendorf MasterMix (Eppendorf AG; Hamburg, Germany) and 0.2 µM of each primer. Due to the bidirectional nature of the cloning, a gene-specific primer (RS42) was paired with either vector-specific primer RS7 (Integrated DNA Technologies) or vector-specific primer RS8 (Integrated DNA Technologies) to determine the insert orientation.

Primers Used in PCR Screen for Positive Clones

Name	Primer Sequence	Description
RS7	CGCCAGGGTTTTCCCAGTCACGAC	M13 forward (-41) primer
RS8	AGCGGATAACAATTTCACACAGG	M13 reverse (-48) primer
RS42	CCTACTCCTCGGAGCATTGCCTCGTA	*SEC61γ* subunit, Arabidopsis. Reverse primer, nested. Spans intron.

PCR amplification was carried out on a PrimusHT thermal cycler (MWG-Biotech AG) using the following thermal cycling conditions:

PCR Screen Thermal Cycling Parameters

Temperature	Time	Number of Cycles
95˚C	5 minutes	1
95˚C 55˚C 72˚C	15 seconds 15 seconds 30 seconds	30
72˚C	7 minutes	1
8˚C	∞	

Aliquots of each reaction (5 µl) were separated by electrophoresis at 10 V/cm in a 1% (w/v) agarose gel containing 0.2 µg/ml ethidium bromide in TAE (40 mM Tris-acetate, 1 mM EDTA).

All transformants identified by the PCR screen as containing positive clones were cultured in 2.5 ml of Luria-Bertani broth containing 50 µg/ml kanamycin. Plasmids were purified from these cultures using the QIAprep Spin Miniprep Kit (Qiagen GmbH; Hilden, Germany) in accordance with manufacturer's instructions.

Sequencing. Double-stranded sequencing of selected clones was performed at the BASF AG Sequencing Facility (Ludwigshafen, Germany). Sequencing data was assembled using Sequencher, version 4.5 (Gene Codes Corporation; Ann Arbor, Michigan).

RESULTS AND DISCUSSION

Messenger RNA was extracted from Cultivance Soybean Event 127, conventional soybean variety Conquista, and Arabidopsis Col-0 leaf tissue and used as a template for RLM-5'-RACE. The RLM-5'-RACE secondary PCR amplification was performed using nested primers to increase yield and specificity of the PCR products. Each template was amplified with the GeneRacer 5' Nested Primer paired with either nested gene-specific primer RS43 or RS42. Nested gene-specific primer RS43, when used in combination with the GeneRacer 5' Nested Primer, was expected to amplify the 5' end of cDNAs derived from endogenous soybean *Sec61γ* subunit transcripts. Nested gene-specific

primer RS42, when used in combination with the GeneRacer 5' Nested Primer, was expected to amplify the 5' end of cDNAs derived from Arabidopsis *SEC61γ* subunit (locus At3g48570) transcripts.

Secondary PCR amplifications using soybean *Sec61γ* subunit-specific primer served as a positive control to confirm the ability to amplify endogenous soybean transcripts in the RLM-5'-RACE experiment. Due to the lack of a published full-length cDNA sequence, it was difficult to calculate an expected size for the endogenous soybean *Sec61γ* subunit secondary PCR product. Using an expressed sequence tag (GenBank accession BM731537) which shows 100% identity to both the primary and secondary PCR primers, the endogenous soybean *Sec61γ* subunit secondary PCR product could be roughly estimated to be 199 bp, taking into account that 30 bp of the PCR product are derived from the GeneRacer RNA Oligo. The soybean positive control lanes in both conventional cultivar Conquista and Cultivance Soybean Event 127 show PCR products with similar intensity at the estimated size for the endogenous soybean *Sec61γ* subunit (Figure 3). This proportional amplification of the positive control indicates that the band observed with Arabidopsis *SEC61γ*-specific primers in Event 127, but not in conventional Conquista, is not due to non-specific background amplification.

Event 127 RLM-5'-RACE secondary PCR products amplified with Arabidopsis *SEC61γ*-specific primer RS42 are not a single distinct band, indicating multiple transcript sizes. The presence of PCR products in the corresponding "minus TAP" control lane indicates that some of these PCR products are derived from partial transcripts. However, because the largest PCR product in this "minus TAP" control lane is clearly smaller than the largest PCR products observed in the corresponding "plus TAP" lane, it is expected that the largest Event 127 secondary PCR products amplified with Arabidopsis *SEC61γ*-specific primers represent full-length transcripts. The largest Event 127 RLM-5'-RACE secondary PCR product amplified with Arabidopsis *SEC61γ*–specific primers is clearly larger than the PCR product amplified from Arabidopsis ecotype Col-0 with Arabidopsis *SEC61γ* -specific primers suggesting a transcriptional fusion in Event 127 (Figure 3).

The Cultivance Soybean Event 127 RLM-5'-RACE secondary PCR products generated with Arabidopsis *SEC61γ* subunit-specific primers were cloned into vector pCR4-TOPO. Twelve transformants were screened for positive clones by colony PCR using a gene-specific primer. Based on the appearance of a PCR product in the colony PCR screen, all twelve colonies were deemed to contain positive clones albeit in differing orientations. Consistent with the smeared band observed in the RLM-5'-RACE secondary PCR, the twelve clones displayed a variety of insert sizes (Figure 4). Plasmid DNA was isolated from all twelve colonies and submitted for double-stranded sequencing.

The two longest RLM-5'-RACE secondary PCR clones were identical. Both 419 bp clones were a chimera of the flanking soybean genomic sequence and the Arabidopsis *SEC61γ* gene (Figure 5). Thus, in Cultivance Soybean Event 127 the Arabidopsis

SEC61γ transcript is actually a transcriptional fusion with a short segment of adjacent soybean flanking sequence.

The longest RLM-5'-RACE cDNA clones contained 89 nucleotides derived from Event 127 flanking soybean genomic sequence, followed by 18 nucleotides of unannotated Arabidopsis genomic sequence (which is presumably a small segment of the native Arabidopsis *SEC61γ* promoter), followed by the Arabidopsis *SEC61γ* full-length cDNA sequence (GenBank accession NM_114716) through the RS42 binding site (Figure 6). It would be expected that the actual transcript in Event 127 continues through the native Arabidopsis *SEC61γ* 3' untranslated region, but that was not addressed in this experiment.

No nucleotide differences were observed between the longest RLM-5'-RACE clones and the corresponding region of the Cultivance Soybean Event 127 flanking sequence and transgene insert once the Arabidopsis *SEC61γ* introns were removed from the genomic sequence to account for mRNA splicing. The fact that both Arabidopsis *SEC61γ* introns 1 and 2 are properly spliced out of the transcript (in agreement with the At3g48570.1 full-length cDNA sequence from The Arabidopsis Information Resource) indicates that the RLM-5'-RACE clones are indeed derived from a transcript rather than inadvertently cloned genomic DNA fragments.

The first "ATG" codon in the longest RLM-5'-RACE cDNA sequence coincides with the predicted translation initiation site of the Arabidopsis *SEC61γ* subunit. The entire coding sequence of the Arabidopsis *SEC61γ* subunit through the stop codon is included in the Cultivance Soybean Event 127 RLM-5'-RACE cDNA sequence. As there are no changes at the nucleotide level, the predicted protein sequence of the Arabidopsis *SEC61γ* subunit in Cultivance Soybean Event 127 does not differ from that in Arabidopsis.

CONCLUSIONS

The 5' end of the Arabidopsis *SEC61γ* subunit transcript in Cultivance Soybean Event 127 was determined by RLM-5'-RACE. The Arabidopsis *SEC61γ* subunit transcript in Event 127 is actually a transcriptional fusion with a short segment (89 nucleotides) of adjacent soybean flanking sequence in the longest RLM-5'-RACE clone identified in this study. However, the first "ATG" codon in the longest RLM-5'-RACE cDNA sequence coincides with the predicted translation initiation site of the Arabidopsis *SEC61γ* subunit. As there are no changes at the nucleotide level, the predicted protein sequence of the Arabidopsis *SEC61γ* subunit in Cultivance Soybean Event 127 does not differ from that in Arabidopsis.

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Reported by: [redacted] Feb. 26, 2007
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[redacted] 26 Feb 2007
Senior Manager, Regulatory Science — Date

Submitted by: [redacted] 26 Feb 2007
Regulatory Affairs Manager — Date

REFERENCES

Sathasivan, K., Haughn, G. W., and Murai, N. (1990) Nucleotide sequence of a mutant acetolactate synthase gene from an imidazolinone-resistant *Arabidopsis thaliana* var. Columbia. Nucl. Acids Res. 18:2188.

Shen, Y. (2007) Molecular characterization of Cultivance Soybean Event 127. BASF Plant Science Report No. BPS-001-06.

Figure 1. Plasmid pAC321

The pAC321 PvuII fragment containing the *AHASL* 5' UTR, *csr1-2* coding sequence, and *AHASL* 3' UTR was used for transformation to produce Cultivance Soybean Event 127. The *csr1-2* coding sequence contains a single mutation (Ser653Asn) in the Arabidopsis acetohydroxyacid synthase large subunit which confers resistance to imidazolinone herbicides (Sathasivan *et al.*, 1990).

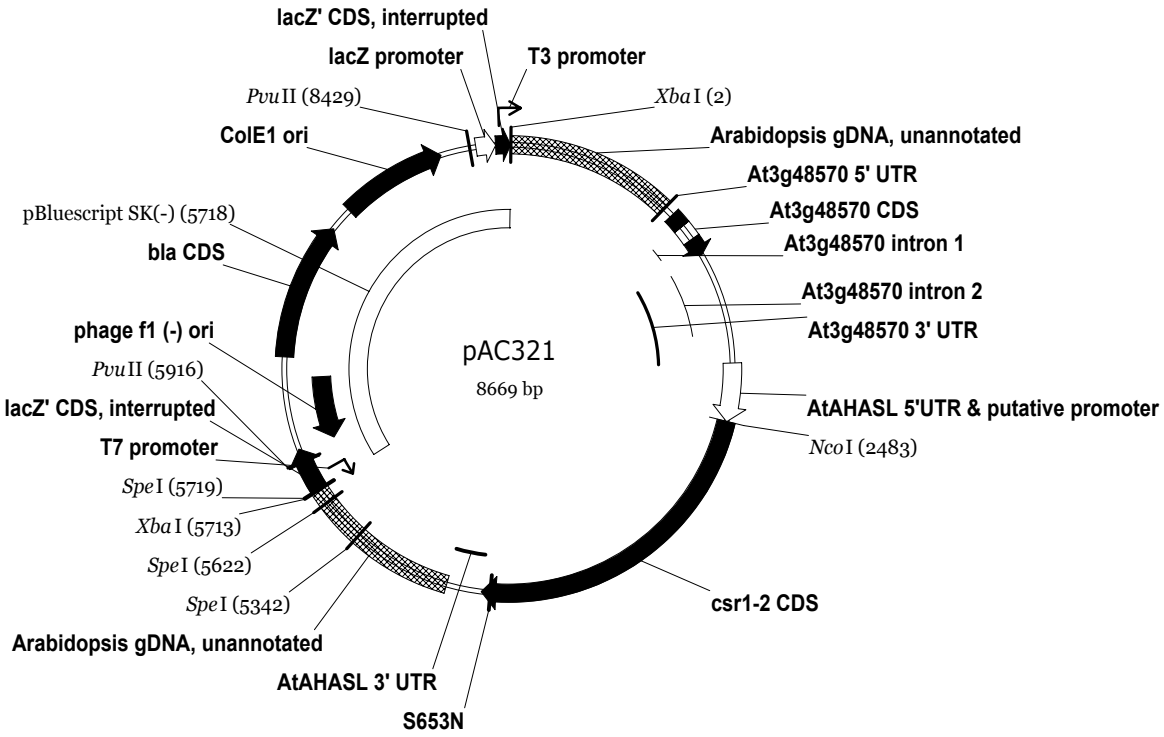
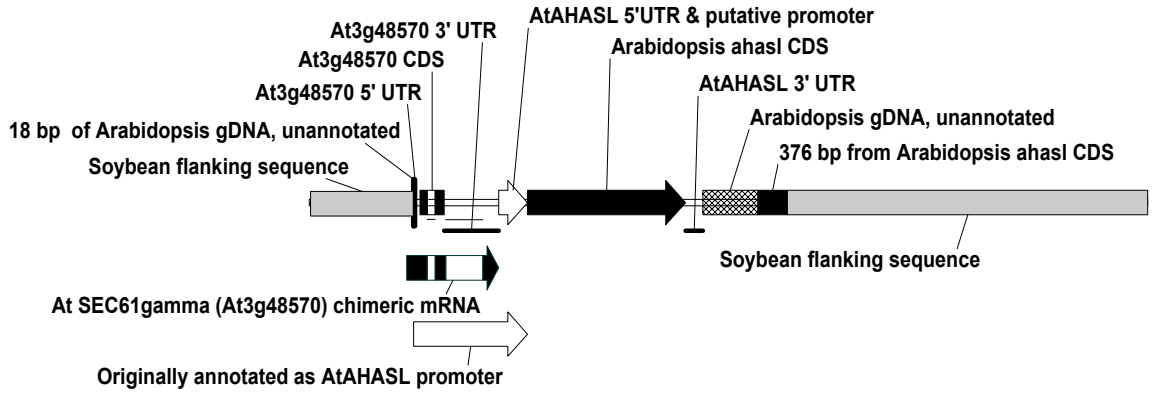


Figure 2. Graphical Representation of Transgene Integration Site in Cultivance Soybean Event 127

Graphical representation of the Cultivance Soybean Event 127 transgene integration site is pictured in Panel A. Panel B depicts a more detailed view of the region of the Event 127 transgene integration site containing the majority of the Arabidopsis *SEC61γ* gene. The chimeric Arabidopsis *SEC61γ* subunit messenger RNA shown below is based on the assumption that the 3' end of this chimeric transcript in Event 127 is identical to the 3' end of the native Arabidopsis *SEC61γ* subunit message.

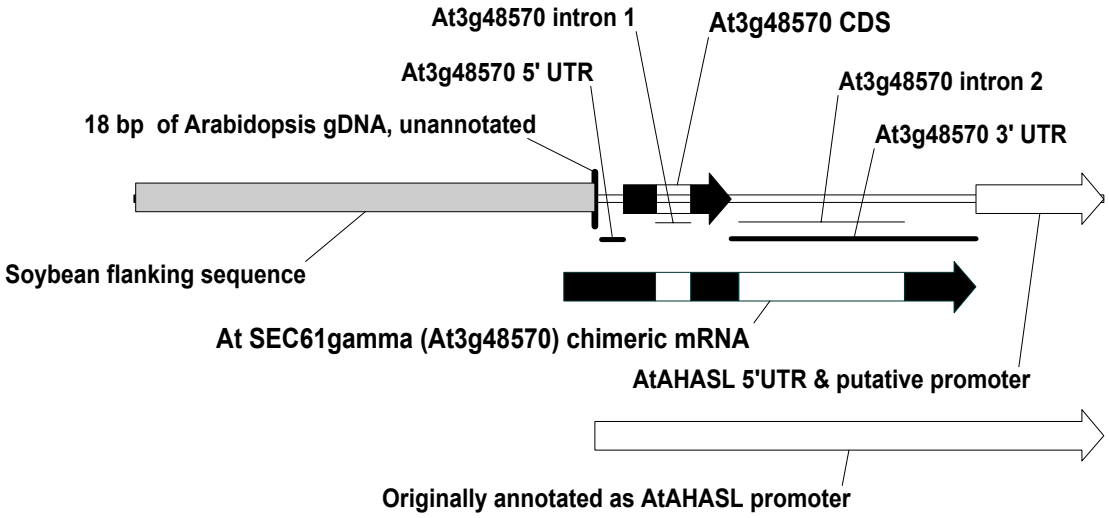
Panel A



Cultivance Soybean Event 127 transgene insert

10656 bp

Panel B

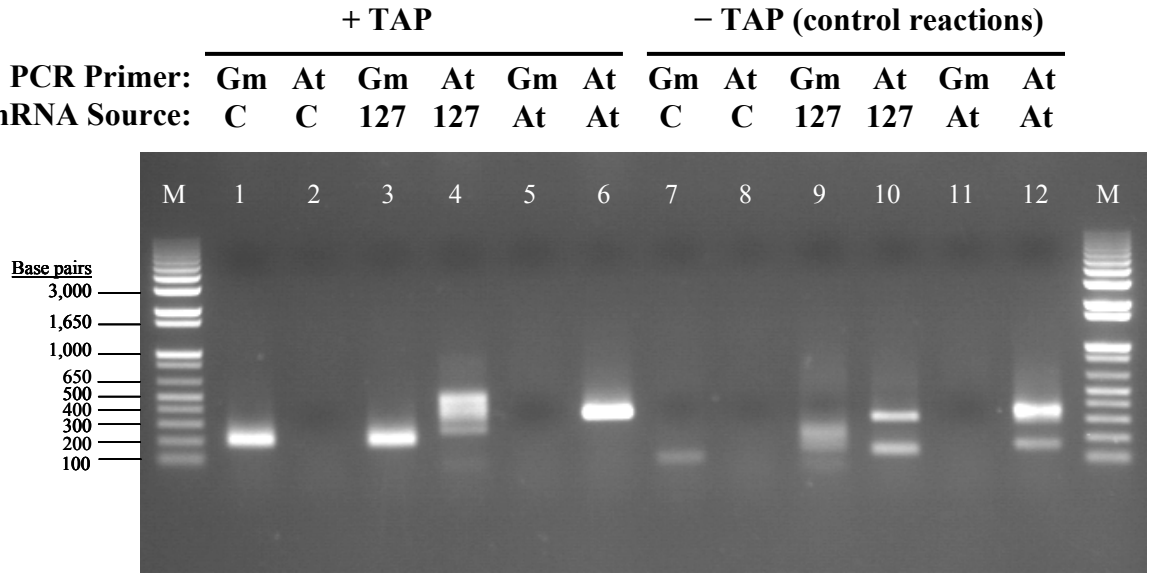


Cultivance Soybean Event 127 trangene insert. Detailed view

2761 bp

Figure 3. RLM-5'-RACE Secondary PCR

Messenger RNA extracted from Cultivance Soybean Event 127 ("127" in figure below), conventional soybean variety Conquista ("C" in figure below), and Arabidopsis ecotype Col-0 ("At" in figure below) leaf tissue was used as a template for RLM-5'-RACE. In the RLM-5'-RACE secondary PCR, the GeneRacer 5' Nested Primer (Invitrogen) was paired with either a primer expected to specifically amplify endogenous soybean *Sec61γ* ("Gm" in figure below) or a primer expected to specifically amplify Arabidopsis *SEC61γ* ("At" in figure below). Lanes 7 through 12 are identical to lanes 1 to 6, respectively, with the exception that lanes 7 through 12 were not treated with tobacco acid pyrophosphatase (TAP) during the RLM-5'-RACE procedure. Samples in lanes 7 through 12 serve as controls: any products observed in these lanes are derived from non-full-length transcripts. Lanes labeled "M" each contain 700 ng of 1 kb Plus DNA Ladder (Invitrogen).



Summary of Expected and Observed Results for RLM-5'-RACE Secondary PCR

Lane in Gel	mRNA Source	Gene-Specific Secondary PCR Primer	Expected Band Size	Expected Result Observed?
1	Conquista leaf	*Sec61γ*, soybean	~199 bp[a]	yes
2	Conquista leaf	*SEC61γ*, Arabidopsis	none	yes
3	Event 127 leaf	*Sec61γ*, soybean	~199 bp[a]	yes
4	Event 127 leaf	*SEC61γ*, Arabidopsis	?	smeared band
5	Arabidopsis leaf	*Sec61γ*, soybean	none	yes
6	Arabidopsis leaf	*SEC61γ*, Arabidopsis	342 bp[b]	yes

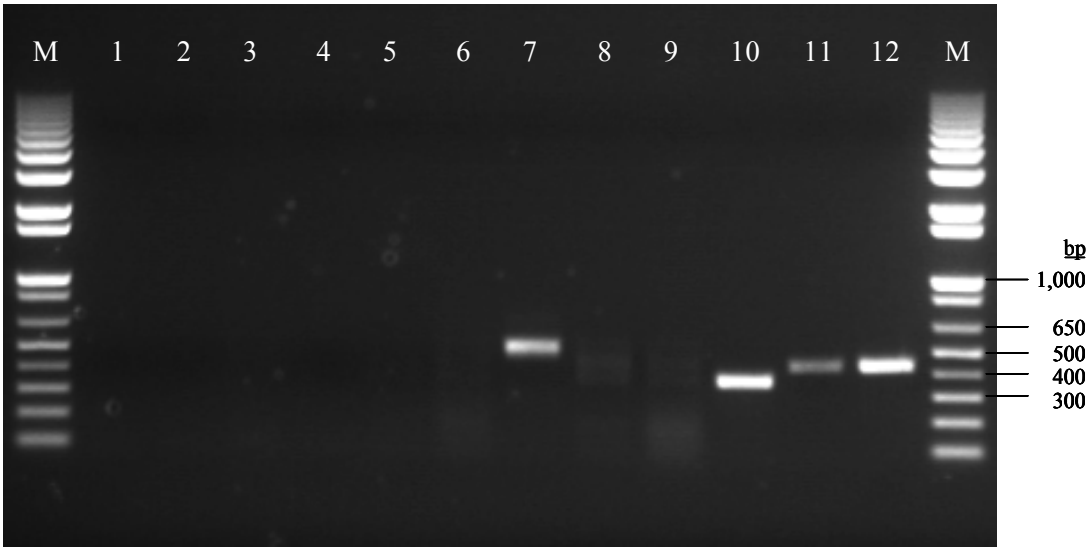
[a]This "expected" band size is estimated: 30 bp are derived from the GeneRacer RNA Oligo and an estimated 169 bp are derived from soybean *Sec61γ*.

[b]A 342 bp band is expected: 30 bp are derived from the GeneRacer RNA Oligo and 312 bp are derived from Arabidopsis *SEC61γ*.

Figure 4. Cloning of RLM-5'-RACE PCR Products: PCR Screen of Transformants

The Cultivance Soybean Event 127 RLM-5'-RACE secondary PCR products generated with Arabidopsis *SEC61γ* subunit-specific primers were cloned into vector pCR4-TOPO. Twelve transformants were screened for positive clones by colony PCR. Due to the bidirectional nature of the cloning, a gene-specific primer (RS42) was paired with either vector-specific primer RS7 [M13 forward (-41) primer] or vector-specific primer RS8 [M13 reverse (-48) primer] to determine the insert orientation. Lanes 1 through 12 in Panels A and B each represent an individual colony. Colonies 1 through 12 were screened by colony PCR using primers RS42 and RS7 (Panel A) and primers RS42 and RS8 (Panel B). Lanes labeled "M" each contain 700 ng of 1 kb Plus DNA Ladder (Invitrogen).

Panel A



Panel B

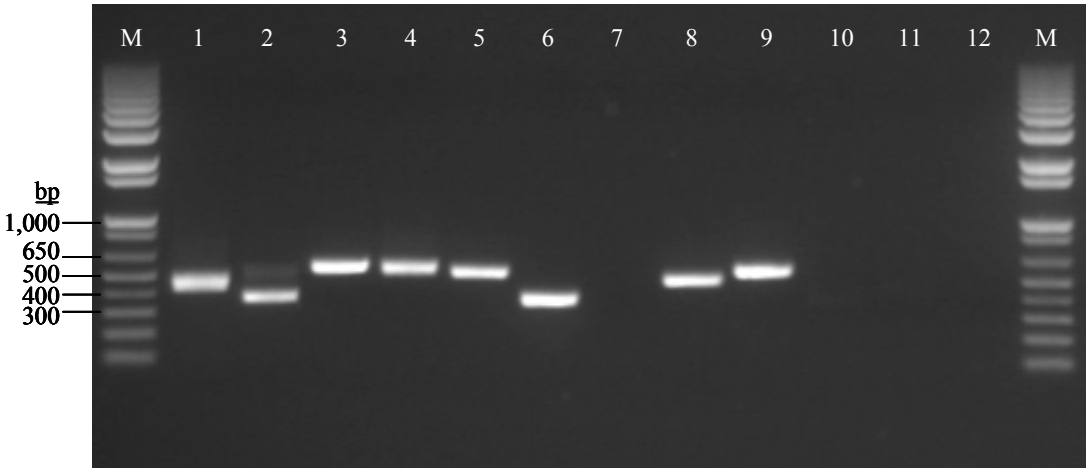
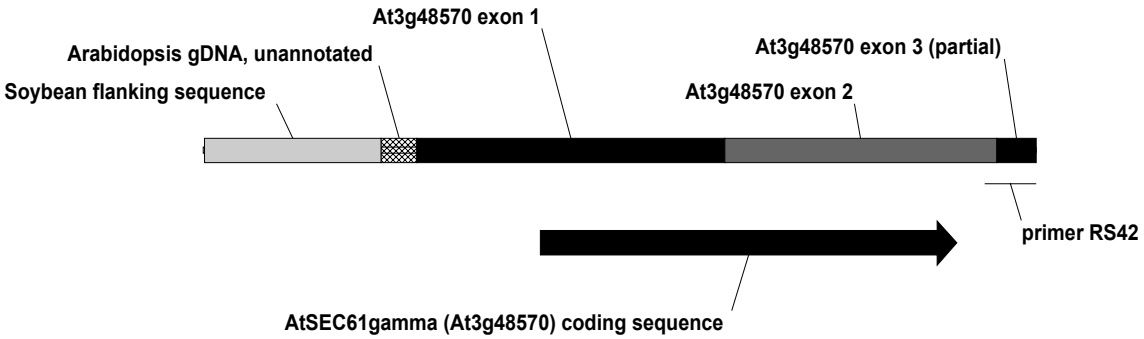


Figure 5. Graphical Representation of Longest RLM-5'-RACE Clone

Graphical representation of the longest RLM-5'-RACE clone is shown below with various regions of the linear fragment representing the cDNA shaded to indicate their origin. The first "ATG" codon in this cDNA sequence coincides with the predicted translation initiation site of the Arabidopsis *SEC61γ* subunit. The location of the Arabidopsis *SEC61γ* subunit coding sequence is indicated by an arrow in the diagram below.



Event 127 RLM-5'-RACE longest clone

419 bp

Figure 6. Sequence of Longest RLM-5'-RACE Clone

Sequence of the longest RLM-5'-RACE clone with the corresponding deduced amino acid sequence is shown below. The origin of each region of the transcript is annotated above (or in the case of primer RS42, below) the cDNA sequence.