syngenta

Event SYHT0H2 Soybean

Insert Sequence Analysis

Final Report

DATA REQUIREMENTS: Not Applicable

AUTHOR: [redacted]

STUDY COMPLETION DATE: May 17, 2012

PERFORMING LABORATORY:
Syngenta Crop Protection, LLC
Product Safety
3054 East Cornwallis Road
Research Triangle Park, NC 27709-2257 USA

SYNGENTA STUDY NO.:
Report Number: TK0059645
Task Number: TK0059645

SUBMITTER:
Syngenta Seeds, Inc.
3054 East Cornwallis Road
Post Office Box 12257
Research Triangle Park, NC 27709-2257 USA

SPONSOR:
Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

VOLUME 1 OF 2 OF STUDY

STATEMENTS OF DATA CONFIDENTIALITY CLAIMS

The following statement applies to submissions to the United States Environmental Protection Agency (US EPA).

No Claim of Confidentiality

A claim of confidentiality is being made for information contained in this report on the basis of its falling within the scope of Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Section 10 (d) (1) (A), (B), or (C). The information claimed confidential has been removed to a confidential volume and is cited by cross reference number in the body of this volume.

Company: *Syngenta Seeds, Inc.*

Company Representative:

[redacted] May 16, 2012

Date

Manager, Regulatory Affairs

These data are the property of Syngenta Seeds, Inc. and, as such, are considered to be confidential for all purposes other than compliance with the regulations implementing FIFRA Section 10. Submission of these data in compliance with FIFRA does not constitute a waiver of any right to confidentiality that may exist under any other provision of common law or statute or in any other country.

The following statement applies to submissions to regulatory agencies and other competent authorities other than the US EPA and all other viewers.

This Document Contains Confidential Business Information

This document contains information that is proprietary to Syngenta and, as such, is considered to be confidential for all purposes other than compliance with the relevant registration procedures.

Without the prior written consent of Syngenta, this information may (i) not be used by any third party including, but not limited to, any other regulatory authority for the support of regulatory approval of this product or any other product, and (ii) not be published or disclosed to any third party including, but not limited to, any authority for the support of regulatory approval of any products.

Its submission does not constitute a waiver of any right to confidentiality that may exist in any other country.

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with the relevant provisions of Good Laboratory Practice Standards (40 CFR Part 160, US EPA 1989) pursuant to the Federal Insecticide, Fungicide, and Rodenticide Act with the following exceptions:

- The genomic deoxyribonucleic acid used in this study was not extracted under protocol.
- Confirmatory sequencing analysis prior to shipping the DNA samples to GENEWIZ was not conducted under Good Laboratory Practice.

Study Director:

[redacted] _____ May 17, 2012
Date

Technical Expert, Product Safety
Syngenta Crop Protection, LLC

Submitted by:

[redacted] _____ May 16, 2012
Date

Manager, Regulatory Affairs
Syngenta Seeds, Inc.
3054 East Cornwallis Road
Post Office Box 12257
Research Triangle Park, NC 27709-2257 USA

Sponsor:

[redacted] _____ May 17, 2012
Date

Technical Leader, Product Safety
Syngenta Crop Protection, LLC
3054 East Cornwallis Road
Post Office Box 12257
Research Triangle Park, NC 27709-2257 USA

QUALITY ASSURANCE STATEMENT

Study Title: Event SYHT0H2 Soybean Insert Sequence Analysis

Study Director: [redacted]

Study Number: TK0059645

Pursuant to Good Laboratory Practice Regulations (40 CFR Part 160), this statement verifies that the aforementioned study was inspected and/or audited and the findings reported to Management and to the Study Director by the Quality Assurance Unit on the dates listed below.

Inspection/Audit Type	Inspection/Audit Dates	Reporting Date
Audit Protocol	July 21, 2011	July 21, 2011
Inspect Analytical	August 11, 2011	August 12, 2011
Audit Final Report (1st Audit)	March 23 & 26 – 27, 2012	March 30, 2012
Audit Final Report (2nd Audit)	May 15, 2012	May 15, 2012

Please reference the analytical phase report for additional audit/inspection dates.

Prepared by: [redacted] Date: May 15, 2012

Staff Quality Assurance Auditor
Quality Assurance Unit
Syngenta

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
[redacted]	Study Director, Syngenta Crop Protection, LLC Principal Investigator, GENEWIZ, Inc. Technical Scientist, GENEWIZ, Inc.

Study Dates

Study initiation date:	July 21, 2011
Experimental start date:	July 27, 2011
Experimental termination date:	January 17, 2012

Records Retention

Raw data, the original copy of this report, and other relevant records are archived at Syngenta, 3054 East Cornwallis Road, Research Triangle Park, NC 27709-2257 USA. Facility records generated at GENEWIZ are archived at GENEWIZ's archives.

Additional Test Site

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080 USA

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

avhppd-03	*p*-hydroxyphenylpyruvate dioxygenase gene derived from oat
AvHPPD-03	*p*-hydroxyphenylpyruvate dioxygenase enzyme encoded by *avhppd-03*
bp	base pair
CBI	Confidential Business Information
CTAB	cetyltrimethyl ammonium bromide
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
EDTA	ethylenediaminetetraacetic acid
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FMV	figwort mosaic virus
g	gram
HPPD	*p*-hydroxyphenylpyruvate dioxygenase enzyme
M	molar
ml	milliliter
mM	millimolar
pat	phosphinothricin acetyltransferase gene
PAT	phosphinothricin acetyltransferase enzyme
PCR	polymerase chain reaction
S	Svedberg unit
spec	spectinomycin resistance gene
T-DNA	transferred deoxyribonucleic acid
tris	2-amino-2-(hydroxymethyl)-1,3-propanediol
US EPA	United States Environmental Protection Agency
v.	version
v/v	volume to volume
w/v	weight to volume
µg	microgram
× *g*	times gravity

1.0 EXECUTIVE SUMMARY

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide glufosinate, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

The purpose of this study was to determine the deoxyribonucleic acid (DNA) sequence of the SYHT0H2 insert and to assess the organization of the functional elements and the presence of any rearrangements, deletions, and/or base pair changes within the SYHT0H2 insert.

Nine overlapping fragments that cover the entire SYHT0H2 insert were amplified from genomic DNA extracted from SYHT0H2 soybean using polymerase chain reaction. The fragments were cloned, and the sequences of the clones were assembled to generate a consensus sequence for the SYHT0H2 insert. This sequence was then compared with the sequence of the transferred DNA (T-DNA) in plasmid pSYN15954, the transformation plasmid used to create SYHT0H2 soybean.

Comparison of the SYHT0H2 insert sequence with the transformation plasmid pSYN15954 T-DNA sequence showed that the SYHT0H2 insert consists of two inverted and truncated copies of the pSYN15954 T-DNA centered on the right border proximal regions. The two copies are truncated at their right border. The 5′ copy lacks the right border, the entire *avhppd-03* cassette, a portion of the 35S promoter, and the left border. The 3′ copy lacks the right border, the figwort mosaic virus enhancer and a portion of the 35S enhancer from the *avhppd-03* cassette, and the left border. In addition, a 44 base pair DNA sequence that has similarity to the gene *avhppd-03* is located between the two copies. Finally, there is a 17 base pair DNA insertion located in the 35S promoter of the 3′ copy. The last 15 base pairs of the 17 base pair DNA insertion are a duplication of the sequence located just upstream of that insertion.

2.0 INTRODUCTION

Soybean (*Glycine max* [L.] Merrill) has been genetically modified to express the novel genes *avhppd-03* derived from oat (*Avena sativa* L.) and *pat* from *Streptomyces viridochromogenes*. The gene *avhppd-03* encodes a *p*-hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, designated AvHPPD-03, that catalyzes the formation of homogentisic acid, the aromatic precursor in plastoquinone and vitamin E biosynthesis. In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in transgenic Event SYHT0H2 soybean plants confers a mesotrione-tolerance phenotype. The gene *pat* encodes the enzyme phosphinothricin acetyltransferase (PAT), which inactivates the herbicide glufosinate, an inhibitor of glutamine synthetase, an enzyme in the nitrogen assimilation pathway. Expression of *pat* confers a glufosinate-tolerance phenotype, which was used as a selectable marker in the development of SYHT0H2 soybean.

The purpose of this study was to determine the deoxyribonucleic acid (DNA) sequence of the SYHT0H2 insert and to assess the organization of the functional elements and the presence of any rearrangements, deletions, and/or base pair changes within the SYHT0H2 insert.

Due to the complexity of this insert, the genomic DNA was digested with a restriction enzyme prior to polymerase chain reaction (PCR) amplification to prevent the formation of secondary structures such as hairpins during the PCR denaturation and renaturation cycles. Different restriction enzymes were used in separate reactions to facilitate the synthesis of overlapping PCR fragments that cover the entire SYHT0H2 insert. The PCR fragments were cloned, and the sequences of the clones were assembled to generate a consensus sequence for the SYHT0H2 insert. This sequence was then compared with the sequence of the T-DNA in plasmid pSYN15954, the transformation plasmid used to create SYHT0H2 soybean.

3.0 MATERIALS AND METHODS

3.1 Genetic Elements in Plasmid pSYN15954

Table 1 lists the genetic elements in the transformation plasmid pSYN15954 and their descriptions (including size in base pairs [bp] and position within the plasmid). Elements expected to be transferred to the plant cell and integrated into the plant genome during T-DNA transfer are categorized by the gene cassette in which they are contained. The elements of the plasmid necessary for its replication and selection in different bacterial hosts are categorized as plasmid backbone. These elements are not expected to be transferred to the plant cell and integrated into the plant genome during T-DNA transfer. The left and right borders are categorized as border regions since only a portion of each border is expected to be integrated into the plant genome (Tzfira *et al*. 2004). Figure 1 shows a map of plasmid pSYN15954.

TABLE 1 Genetic Elements in Plasmid pSYN15954

Genetic element	Size (bp)	Position	Description
***avhppd-03* cassette**			
Intervening sequence	282	26 to 307	Intervening sequence with restriction sites used for cloning.
FMV enhancer	194	308 to 501	Figwort mosaic virus (FMV) transcriptional enhancer region (similar to Accession Number X06166.1 [NCBI 2012]) which increases gene expression (Maiti *et al.* 1997).
Intervening sequence	6	502 to 507	Intervening sequence with restriction sites used for cloning.
35S enhancer	293	508 to 800	Cauliflower mosaic virus 35S transcriptional enhancer region (Ow *et al.* 1987).
Intervening sequence	20	801 to 820	Intervening sequence with restriction sites used for cloning.
SMP promoter	39	821 to 859	Synthetic minimal plant promoter (SMP) including the TATA box, an adenine-rich sequence involved in transcription initiation, from the cestrum yellow leaf curling virus promoter (Stavolone *et al*. 2003a), linked to a sequence taken from the region that is 3′ to the TATA box of the 35S promoter (Ow *et al.* 1987).
Intervening sequence	5	860 to 864	Intervening sequence with restriction sites used for cloning.
TMV enhancer	68	865 to 932	The 5′ non-coding leader sequence (called omega) from tobacco mosaic virus (TMV) (Gallie *et al.* 1987) functions as a translational enhancer in plants (Gallie 2002).
Intervening sequence	3	933 to 935	Intervening sequence with restriction sites used for cloning.
avhppd-03	1320	936 to 2255	The gene *avhppd-03*, derived from oat, encodes an AvHPPD-03 enzyme. This enzyme catalyzes the formation of homogentisic acid, the aromatic precursor of plastoquinone and vitamin E biosynthesis (Matringe *et al.* 2005). In comparison with the native soybean HPPD, the AvHPPD-03 isozyme from oat has lower binding affinity for mesotrione, an herbicide that inhibits HPPD. Expression of *avhppd-03* in plant cells confers a mesotrione-tolerance phenotype.
Intervening sequence	16	2256 to 2271	Intervening sequence with restriction sites used for cloning.
NOS terminator	253	2272 to 2524	Terminator sequence from the nopaline synthase (NOS) gene of *Agrobacterium. tumefaciens* (Accession Number V00087.1 [NCBI 2012]). Provides a polyadenylation site (Depicker *et al*. 1982).
Intervening sequence	8	2525 to 2532	Intervening sequence with restriction sites used for cloning.
***pat-03-01* cassette**			
35S promoter	521	2533 to 3053	Promoter region of cauliflower mosaic virus (Ow *et al.* 1987).
Intervening sequence	24	3054 to 3077	Intervening sequence with restriction sites used for cloning.

NCBI = National Center for Biotechnology Information

TABLE 1 Genetic Elements in Plasmid pSYN15954 (Continued)

Genetic element	Size (bp)	Position	Description
pat-03-01	552	3078 to 3629	*Streptomyces viridochromogenes* strain Tü494 gene encoding the selectable marker PAT. The native coding sequence (Wohlleben *et al*. 1988) was codon-optimized for enhanced expression. The synthetic *pat-03-01* gene was obtained from AgrEvo, Germany. PAT confers resistance to herbicides containing glufosinate (phosphinothricin).
Intervening sequence	33	3630 to 3662	Intervening sequence with restriction sites used for cloning.
NOS terminator	253	3663 to 3915	Terminator sequence from the NOS gene of *A. tumefaciens* (Accession Number V00087.1 [NCBI 2012]). Provides a polyadenylation site (Depicker *et al*. 1982).
Intervening sequence	8	3916 to 3923	Intervening sequence with restriction sites used for cloning.
***pat-03-02* cassette**			
CMP promoter	654	3924 to 4577	Promoter and leader sequence from the cestrum yellow leaf curling virus, similar to Accession Number AF364175.3 (NCBI 2012) (Stavolone *et al*. 2003b).
Intervening sequence	5	4578 to 4582	Intervening sequence with restriction sites used for cloning.
TMV enhancer	68	4583 to 4650	The 5′ non-coding leader sequence (called omega) from tobacco mosaic virus (Gallie *et al.* 1987) functions as a translational enhancer in plants (Gallie 2002).
Intervening sequence	10	4651 to 4660	Intervening sequence with restriction sites used for cloning.
pat-03-02	552	4661 to 5212	*S. viridochromogenes* strain Tü494 gene encoding the selectable marker PAT. The native coding sequence (Wohlleben *et al*. 1988) was codon-optimized for enhanced expression and altered to remove restriction sites. PAT confers resistance to herbicides containing glufosinate (phosphinothricin).
Intervening sequence	28	5213 to 5240	Intervening sequence with restriction sites used for cloning.
NOS terminator	253	5241 to 5493	Terminator sequence from the nopaline synthase gene of *A. tumefaciens* (Accession Number V00087.1 [NCBI 2012]). Provides a polyadenylation site (Depicker *et al*. 1982).
Intervening sequence	77	5494 to 5570	Intervening sequence with restriction sites used for cloning.
Border Region			
Left Border	25	5571 to 5595	Left border region of T-DNA from *A. tumefaciens* nopaline Ti-plasmid (Accession Number J01825.1 [NCBI 2012]). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Zambryski *et al*. 1982).
Plasmid backbone			
Intervening sequence	349	5596 to 5944	Intervening sequence with restriction sites used for cloning.
spec	789	5945 to 6733	Aminoglycoside adenylyltransferase gene, *aadA*, from *Escherichia coli* transposon Tn7 (similar to Accession Number X03043.1 [NCBI 2012]). Confers resistance to streptomycin and spectinomycin and is used as a bacterial selectable marker (Fling *et al*. 1985).

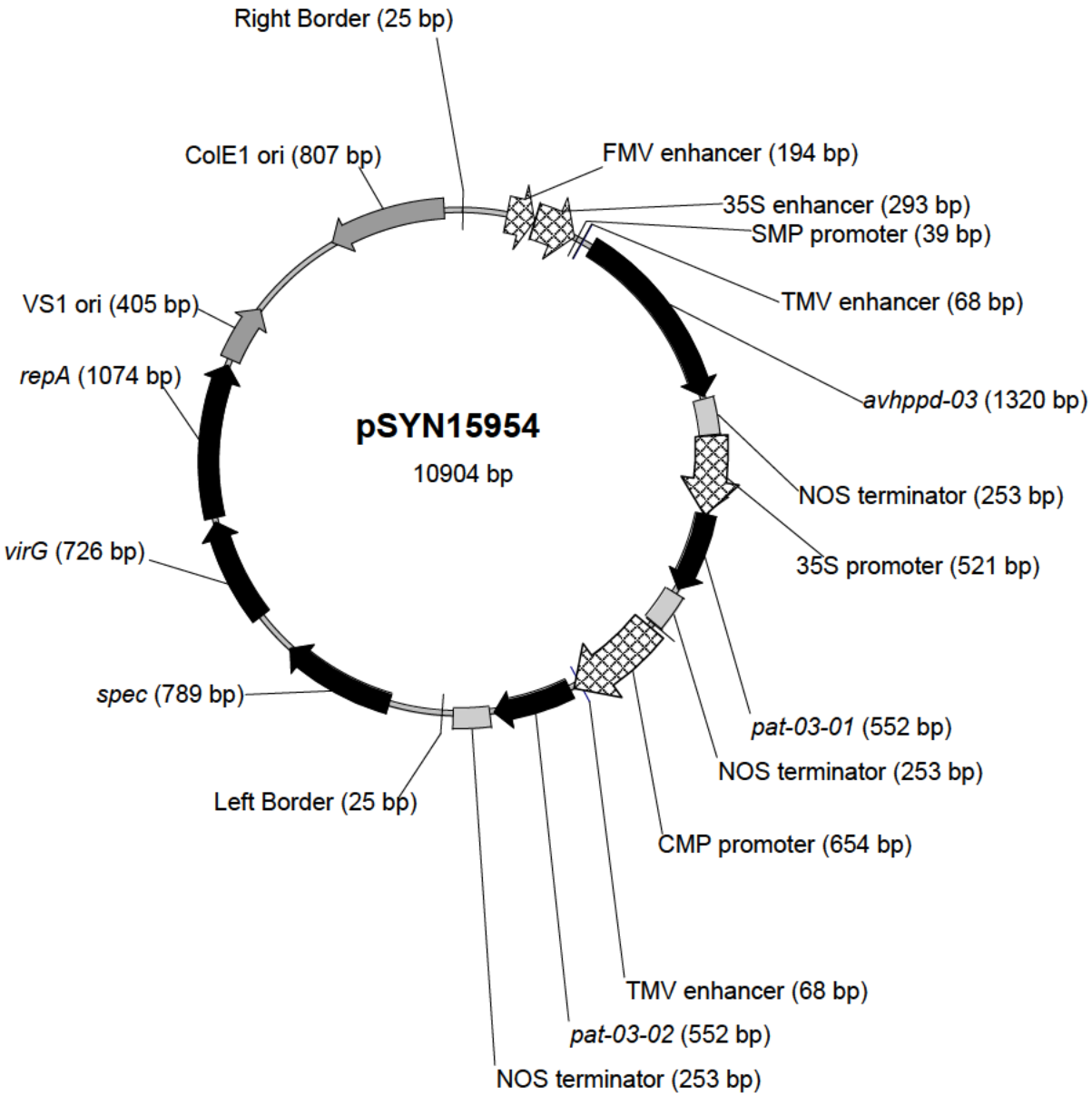
CMP = Cestrum yellow leaf curling virus promoter
NCBI = National Center for Biotechnology Information

TABLE 1 Genetic Elements in Plasmid pSYN15954 (Continued)

Genetic element	Size (bp)	Position	Description
Intervening sequence	299	6734 to 7032	Intervening sequence with restriction sites used for cloning.
virG	726	7033 to 7758	The VirGN54D gene (*virG*) from pAD1289 (similar to Accession Number AF242881.1 [NCBI 2012]). The N54D substitution results in a constitutive virG phenotype. The gene *virG* is part of the two-component regulatory system for the virulence regulon in *A. tumefaciens* (Hansen *et al*. 1994).
Intervening sequence	29	7759 to 7787	Intervening sequence with restriction sites used for cloning.
repA	1074	7788 to 8861	Gene encoding the pVS1 replication protein from *Pseudomonas aeruginosa* (similar to Accession Number AF133831.1 [NCBI 2012]), which is a part of the minimal pVS1 replicon that is functional in Gram-negative, plant-associated bacteria (Heeb *et al*. 2000).
Intervening sequence	42	8862 to 8903	Intervening sequence with restriction sites used for cloning.
VS1 ori	405	8904 to 9308	Consensus sequence for the origin of replication (ori) and partitioning region from plasmid pVS1 of *P. aeruginosa* (Accession Number U10487.1 [NCBI 2012]). Serves as origin of replication in *A. tumefaciens* host (Itoh *et al*. 1984).
Intervening sequence	677	9309 to 9985	Intervening sequence with restriction sites used for cloning.
ColE1 ori	807	9986 to 10792	Origin of replication (similar to Accession Number V00268.1 [NCBI 2012]) that permits replication of plasmids in *E. coli* (Itoh and Tomizawa 1979).
Intervening sequence	112	10793 to 10904	Intervening sequence with restriction sites used for cloning.
Border Region			
Right Border	25	1 to 25	Right border region of T-DNA from *A. tumefaciens* nopaline Ti-plasmid (Accession Number J01826.1 [NCBI 2012]). Short direct repeat that flanks the T-DNA and is required for the transfer of the T-DNA into the plant cell (Wang *et al*. 1984).

NCBI = National Center for Biotechnology Information
ori = origin of replication
repA = replication gene
virG = part of the two-component regulatory system for the virulence regulon in *A.tumefaciens*

FIGURE 1 Map of Plasmid pSYN15954



CMP = cestrum yellow leaf curling virus promoter
FMV = figwort mosaic virus
NOS = nopaline synthase
ori = origin of replication
repA = replication gene
SMP = synthetic minimal plant promoter
TMV = tobacco mosaic virus
virG = part of the two-component regulatory system for the virulence regulon in *A.tumefaciens*

3.2 Test Substance

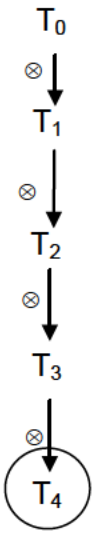
The test substance for this study was SYHT0H2 soybean seed in the genetic background 'Jack' (Nickell *et al.* 1990). Table 2 shows the material identification for the test substance. Figure 2 is a pedigree chart illustrating the production of the test substance.

TABLE 2 Test Substance

Seed identification	Material identification
SYHT0H2 T_4 soybean	09SG052316

The test substance was characterized by real-time PCR analysis (Ingham *et al.* 2001) to confirm identity and purity (Burgin 2011).

FIGURE 2 Pedigree Chart for SYHT0H2 Soybean Illustrating the Production of the Test Substance Used in This Study



The transformation recipient line is 'Jack'

T_0 = original transformant
⊗ = self-pollination
The generation used in this study is denoted with a circle.

3.3 Plant Tissue for Genomic DNA Extraction

The test substance seed was grown in a greenhouse located at Syngenta, in Research Triangle Park, NC, USA. Following verification of the plants' identity by real-time PCR analysis, leaf tissue from plants grown from the test substance was pooled into a sampling bag and stored at –80°C ± 10°C (Burgin 2011).

3.4 Genomic DNA Extraction

Genomic DNA was isolated from the pooled leaf tissue using a modification of the method described by Saghai-Maroof *et al*. (1984).

Pooled leaf tissue was ground into a fine powder using a pre-chilled mortar and pestle, with liquid nitrogen, and then placed into a bottle for storage. For each DNA extraction, approximately 5 g of this tissue and 25 ml of pre-warmed cetyltrimethyl ammonium bromide (CTAB) buffer (100 mM 2-amino-2[hydroxymethyl]-1,3-propanediol [tris] pH 8.0, 20 mM ethylenediaminetetraacetic acid [EDTA] pH 8.0, 1.4 M sodium chloride, 2% CTAB [w/v], 0.2% β-mercaptoethanol [v/v]) were combined in a bottle; the sample was then mixed gently and incubated for 73 minutes at 65°C. An equal volume of chloroform:isoamyl alcohol (24:1) was then added, followed by gentle mixing and centrifugation for 10 minutes at 7277 × *g* at room temperature.

The resulting aqueous phase was transferred to a clean container, and 10 µg of ribonuclease per ml of aqueous phase was added. The sample was mixed and incubated for 32 minutes at 37°C ± 2°C. An equal volume of chloroform:isoamyl alcohol (24:1) was then added, followed by gentle mixing and centrifugation for 10 minutes at 7277 × *g* at room temperature. The aqueous phase was collected in a clean bottle, and the DNA was precipitated with a 0.7 volume of isopropanol. The DNA was then pelleted by centrifugation at 291 × *g* for 10 minutes, washed once with 70% (v/v) ethanol, and centrifuged at 7277 × *g* for 10 minutes. The DNA pellet was air-dried and dissolved in pre-warmed 0.1X tris-EDTA.

3.5 DNA Quantitation

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

3.6 Genomic DNA Digestion

Prior to PCR amplification, SYHT0H2 T_4 genomic DNA was digested with the restriction enzymes *Bgl*II, *Ahd*I, *Bsp*EI, and *Bsr*DI in separate reactions.

3.7 PCR Amplification

The digested genomic DNAs were used as a template for the Sigma-Aldrich® JumpStart™ REDAccuTaq® LA DNA Polymerase PCR system to amplify nine overlapping DNA fragments that cover the entire SYHT0H2 insert. The sequence of the PCR primers is shown in Table 3. Table 4 shows the primer pairs used with each of the four digested genomic DNA templates, the resulting PCR products, and the size of the PCR products. Figure 3 shows the location of the nine overlapping PCR-amplified products. Table 5 lists the thermal cycling parameters for PCR amplification of PCR products A-3, B-4, C-6, D-8, E-10 and G-12. Table 6 lists the thermal cycling parameters for PCR amplification of PCR products F-11, H-17 and I-18.

TABLE 3 Primers Used to Amplify the SYHT0H2 Soybean Insert

{Volume 2: Confidential Business Information (CBI) Cross-reference Number 1}

TABLE 4 Digested Genomic DNA Templates and Primer Pairs Used to Amplify Overlapping PCR Products

Template	PCR primer pairs	PCR product name	PCR product size (bp)
Genomic DNA digested with *Ahd*I	Forward: 69s-0H2-F5 Reverse: 60s-0H2-R2	A-3	1219
	Forward: 81s-0H2-F11 Reverse: 58s-0H2-R1	B-4	823
	Forward: 71s-0H2-F6 Reverse: 74s-0H2-R7	C-6	2643
Genomic DNA digested with *Bgl*II	Forward: FE3442 Reverse: 66s-0H2-R3	D-8	2027
	Forward: 67s-0H2-F4 Reverse: FE3443	E-10	2146
Genomic DNA digested with *Bsp*EI	Forward: 75s-0H2-F8 Reverse: 76s-0H2-R8	F-11	384
	Forward: 77s-0H2-F9 Reverse: 78s-0H2-R9	G-12	2219
Genomic DNA digested with *Bsr*DI	Forward: 90s-0H2-F17 Reverse: 84s-0H2-F12	H-17	138
	Forward: 84s-0H2-F12 Reverse: 83s-0H2-R12	I-18	138

FIGURE 3 Location of the Nine Overlapping PCR Products Amplified from SYHT0H2 Soybean and Used to Determine the Insert Sequence

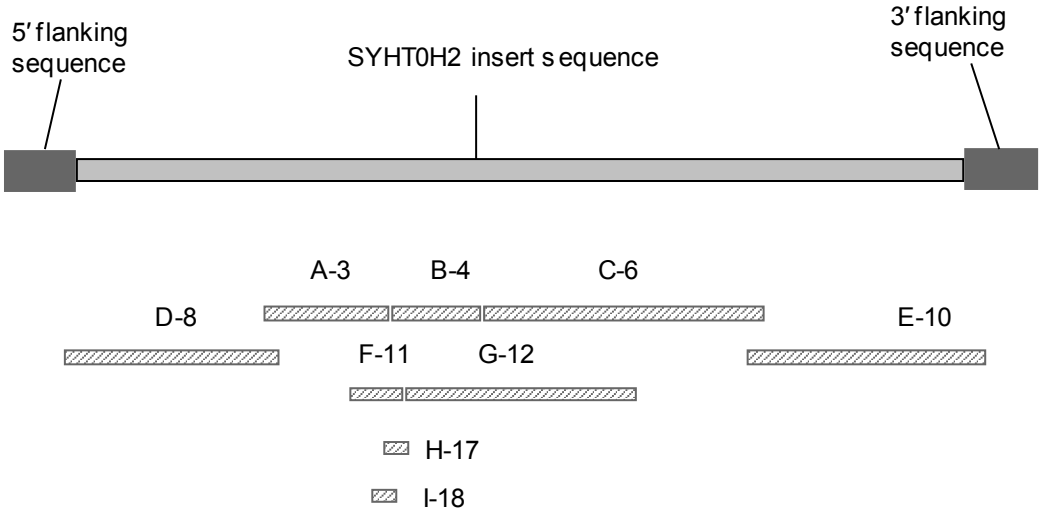


TABLE 5 Thermal Cycling Parameters for PCR Amplification of PCR Products A-3, B-4, C-6, D-8, E-10 and G-12

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	96	30 s	1
B	1	94	15 s	35
	2	60	30 s	
	3	68	3 min	
C	1	68	10 min	1
D	1	4	hold	1

min = minute
s = second

TABLE 6 Thermal Cycling Parameters for PCR Amplification of PCR Products F-11, H-17 and I-18

Cycle	Step	Temperature (°C)	Time	Number of cycles
A	1	96	30 s	1
B	1	94	15 s	35
	2	58	30 s	
	3	68	30 s	
C	1	68	10 min	1
D	1	4	hold	1

min = minute
s = second

The PCR products were cloned into the Invitrogen pCR®4-TOPO® TA vector, and four to six colonies from each cloning reaction were randomly selected and grown. The plasmid DNA from each colony was extracted and digested with the restriction enzyme *Eco*RI to confirm the presence of the expected size insert. Following this confirmation, three DNA clones were then randomly selected, and the presence of the expected insert in each of these was confirmed by single sequencing runs on the ends of the clones using primers located in the TOPO® cloning vector.

3.8 Sequencing

Three individual clones for each of the nine overlapping PCR products were sequenced individually by GENEWIZ, Inc. (South Plainfield, NJ, USA), as reported in Appendix A. The sequences of the clones were analyzed and aligned using Sequence Analysis software v. 5.3 (Applied BioSystems) and Lasergene® software v. 8 (DNASTAR) in order to generate a consensus sequence for each clone. The three clone sequences were aligned to obtain a consensus sequence for each PCR product. Assembly of the nine PCR products' consensus sequences was carried out using Sequencher® v. 4.9 (Gene Codes) in order to generate the final sequence of the SYHT0H2 insert. The SYHT0H2 insert sequence was compared to the sequence of the transformation plasmid pSYN15954 T-DNA using Vector NTI® v. 10.

3.9 Control of Bias

PCR products were cloned, and for each product three colonies were randomly chosen. Any rejected data and the documented reasons for the rejection of those data were retained in the study file.

3.10 Statistical Analysis

No statistical analysis was conducted during this study.

4.0 RESULTS AND DISCUSSION

4.1 Insert Sequencing

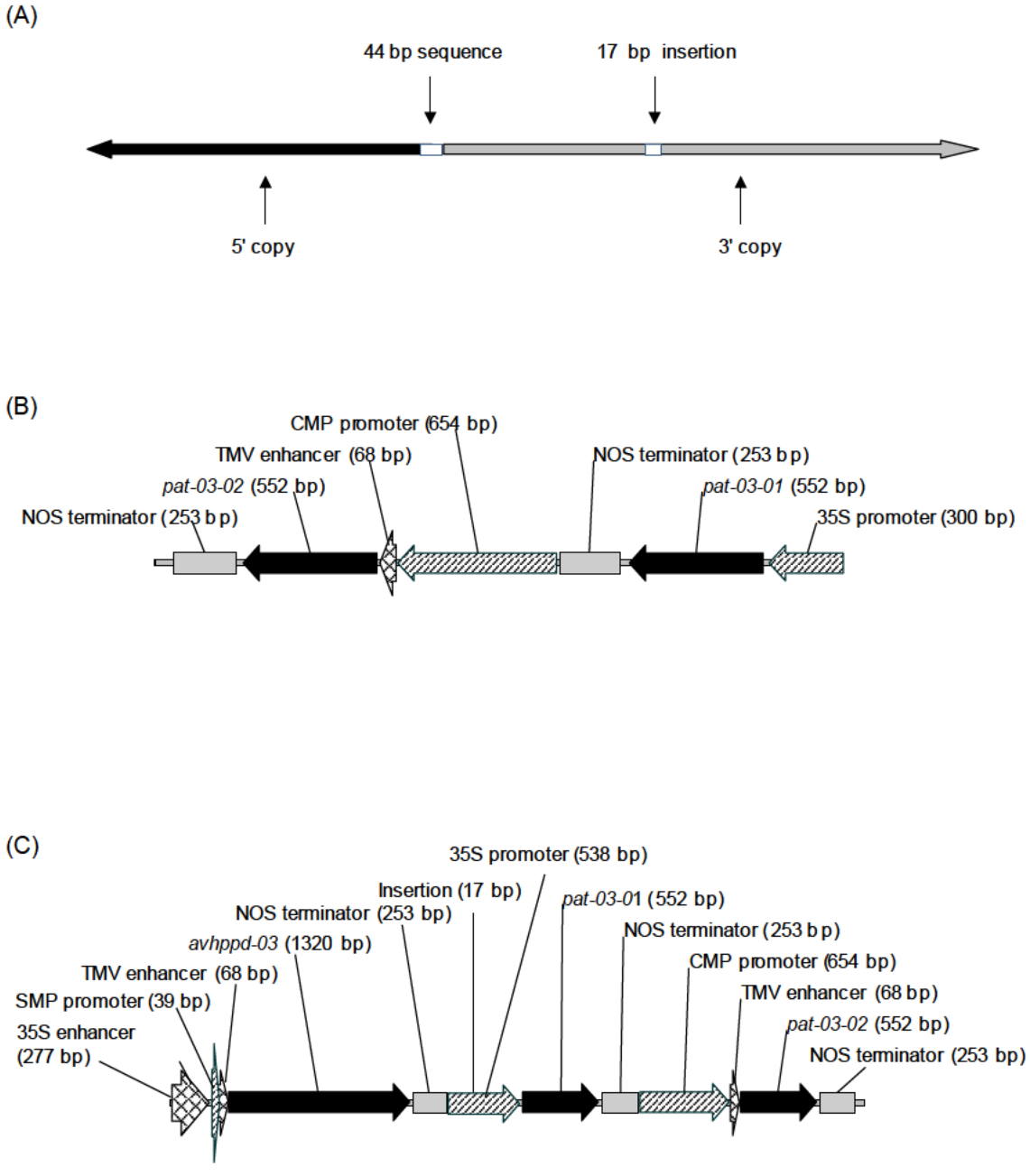
The sequence of the SYHT0H2 insert was determined and was compared to the sequence of the transformation plasmid pSYN15954. This analysis showed that the SYHT0H2 insert consists of two inverted and truncated copies of the pSYN15954 T-DNA centered on the right border proximal regions. These are shown in Figure 4A with a black arrow for the 5′ copy and a grey arrow for the 3′ copy. Both copies are truncated at their right border. The 5′ copy lacks the right border, the entire *avhppd-03* cassette, a portion of the 35S promoter, and the left border (Figure 4B). The 3′ copy lacks the right border, the figwort mosaic virus (FMV) enhancer and a portion of the 35S enhancer from the *avhppd-03* cassette, and the left border (Figure 4C).

The SYHT0H2 insert also contains two additional DNA sequences: the first sequence, which is 44 bp long, is located between the two inverted copies and has similarity to the gene *avhppd-03*; the second sequence is a 17 bp DNA insertion located in the 35S promoter of the 3' copy (Figure 4A). The last 15 bp of the 17 bp DNA insertion are a duplication of the sequence located just upstream of this insertion.

Figure 5A shows the nucleotide alignment of the SYHT0H2 5′ copy with the reverse complement of pSYN15954 T-DNA. Figure 5B shows the nucleotide alignment of the SYHT0H2 3′ copy with the pSYN15954 T-DNA. Figure 5C shows the nucleotide alignment of the 44 bp sequence present between the two inverted copies with the gene *avhppd-03*.

The SYHT0H2 insert sequence in GenBank format is provided in Appendix B.

FIGURE 4 **Organization of the SYHT0H2 Insert: (A) Schematic Representation of the Two Inverted Copies with Location of the Additional DNA Sequences (44 bp Sequence and 17 bp Insert) (B) Elements Present in the 5′ Copy (C) Elements Present in the 3′ Copy.**



Location of the 44 bp sequence within the SYHT0H2 insert sequence is at bp 2817 to bp 2860; location of the 17 bp insert within the SYHT0H2 insert sequence is at bp 5066 to bp 5082.

FIGURE 5 (A) Nucleotide Alignment of the SYHT0H2 Insert 5′ Copy with the pSYN15954 T-DNA Reverse Complement; (B) Nucleotide Alignment of the SYHT0H2 Insert 3′ Copy with the pSYN15954 T-DNA; (C) Nucleotide Alignment of the 44 bp DNA Sequence with the Gene *avhppd-03*

{Volume 2: CBI Cross-reference Number 2}

4.2 Data Quality and Integrity

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

5.0 CONCLUSION

Comparison of the SYHT0H2 insert sequence with the transformation plasmid pSYN15954 T-DNA sequence showed that the SYHT0H2 insert consists of two inverted and truncated copies of the pSYN15954 T-DNA centered on the right border proximal regions. In addition, a 44 bp long DNA sequence that has similarity to the gene *avhppd-03* is located between the two copies. Finally, there is a 17 bp DNA insertion located in the 35S promoter of the 3′ copy; the last 15 bp of the 17 bp DNA insertion are a duplication of the sequence located just upstream of this insertion. Both copies are truncated at their right border. The 5′ copy lacks the right border, the entire *avhppd-03* cassette, part of the 35S promoter and the left border. The 3′ copy lacks the right border, the FMV enhancer and a portion of the 35S enhancer from the *avhppd-03* cassette, and the left border.

6.0 REFERENCES

Burgin K. 2011. *Event SYHT0H2 Soybean: Test and Control Substance Characterization of T_4, T_5, and T_6 Generations and 'Jack' Soybean*. Report No. TK0055856. Research Triangle Park, NC: Syngenta Crop Protection, LLC

Depicker A, Stachel S, Dhaese P, Zambryski P, Goodman HM. 1982. Nopaline synthase: Transcript mapping and DNA sequence. *J Mol Appl Genet* 1:561–573.

Fling ME, Kopf J, Richards C. 1985. Nucleotide sequence of the transposon Tn7 gene encoding an aminoglycoside-modifying enzyme, 3′ (9)-*O*-nucleotidyltransferase. *Nucleic Acids Res* 13:7095–7106.

Gallie DR, Sleat DE, Watts JW, Turner PC, Wilson TMA. 1987. The 5′-leader sequence of tobacco mosaic virus RNA enhances the expression of foreign gene transcripts *in vitro* and *in vivo*. *Nucleic Acids Res* 15:3257–3273.

Gallie DR. 2002. The 5′-leader of tobacco mosaic virus promotes translation through enhanced recruitment of eIF4F. *Nucleic Acids Res* 30:3401–3411.

Hansen G, Das A, Chilton M-D. 1994. Constitutive expression of the virulence genes improves the efficiency of plant transformation by *Agrobacterium.* *P Natl Acad Sci USA* 91:7603–7607.

Heeb S, Itoh Y, Nishijyo T, Schnider U, Keel C, Wade J, Walsh U, O'Gara F, Haas D. 2000. Small, table shuttle vectors based on the minimal pVS1 replicon for use in Gram-negative, plant-associated bacteria. *Mol Plant Microbe In* 13:232–237.

Ingham DJ, Beer S, Money S, Hansen G. 2001. Quantitative real-time PCR assay for determining transgene copy number in transformed plants. *BioTechniques* 31:132–140.

Itoh T, Tomizawa J. 1979. Initiation of replication of plasmid ColE1 DNA by RNA polymerase, ribonuclease H and DNA polymerase I. *Cold Spring Harb Sym.* 43:409–417.

Itoh Y, Watson JM, Haas D, Leisinger T. 1984. Genetic and molecular characterization of the *Pseudomonas* plasmid pVS1. *Plasmid* 11:206–220.

Maiti IB, Gowda S, Kiernan J, Ghosh SK, Shepherd RJ. 1997. Promoter/leader deletion analysis and plant expression vectors with the figwort mosaic virus (FMV) full length transcript (FLt) promoter containing single or double enhancer domains. *Transgenic Res* 6:143–156.

Matringe M, Sailland A, Pelissier B, Rolland A, Zink O. 2005. *p*-Hydroxyphenylpyruvate dioxygenase inhibitor-resistant plants. *Pest Manag Sci* 61:269–276.

6.0 REFERENCES (Continued)

NCBI. 2012. Entrez Nucleotide Database. Bethesda, MD: National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health. http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide.

Nickell CD, Noel GR, Thomas DJ, Waller R. 1990. Registration of 'Jack' soybean. *Crop Sci* 30:1365.

Ow DW, Jacobs JD, Howell SH. 1987. Functional regions of the cauliflower mosaic virus 35S RNA promoter determined by use of the firefly luciferase gene as a reporter of promoter activity. *P Natl Acad Sci USA* 84:4870–4874.

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW. 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *P Natl Acad Sci USA* 81:8014–8018.

Stavolone L, Ragozzino A, Hohn T. 2003a. Characterization of Cestrum yellow leaf curling virus: a new member of the family Caulimoviridae. *J Gen Virol* 84:3459–3464.

Stavolone L, Koronova M, Pauli S, Ragozzino A, De Haan P, Milligan S, Lawton K, Hohn T. 2003b. Cestrum yellow leaf curling virus (CmYLCV) promoter: a new strong constitutive promoter for heterologous gene expression in a wide variety of crops. *Plant Mol Biol* 53:703–713.

Tzfira T, Li J, Lacroix B, Citovsky V. 2004. *Agrobacterium* T-DNA integration: molecules and models. *Trends Genet* 20:375–383.

US EPA. 1989. Good Laboratory Practice Standards. 40 CFR Part 160.

Wang K, Herrera-Estrella L, Van Montagu M, Zambryski P. 1984. Right 25 bp terminus sequence of the nopaline T-DNA is essential for and determines direction of DNA transfer from *Agrobacterium* to the plant genome. *Cell* 38:455–462.

Wohlleben W, Arnold W, Broer I, Hillemann D, Strauch E, Pühler A. 1988. Nucleotide sequence of the phosphinothricin *N*-acetyltransferase gene from *Streptomyces viridochromogenes* Tü494 and its expression in *Nicotiana tabacum*. *Gene* 70:25–37.

Zambryski P, Depicker A, Kruger K, Goodman HM. 1982. Tumor induction by *Agrobacterium tumefaciens*: analysis of the boundaries of T-DNA. *J Mol Appl Genet* 1:361–370.

APPENDICES SECTION

APPENDIX A Sequencing Phase Report

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

Amended GLP Phase Report
GW-Syn-001

Event SYHT0H2 Soybean Insert Sequence Analysis

Sponsor

Syngenta Crop Protection, LLC
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419
Phone: 919 281-7277

Study Director

[redacted]
Syngenta Crop Protection, LLC
3054 Cornwallis Rd.
Research Triangle Park, NC 27709
Phone: 919 281-7266

Test Site

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
E-mail: Regulatory@genewiz.com
Tel: 908-222-0711
Fax: 908-333-4511

Principal Investigator

[redacted]
GENEWIZ, Inc.
[redacted]
Tel: 908-222-0711 extension 3248

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

I. Phase Summary

GENEWIZ INC. provided regulatory submission-quality sequencing services for the sample submitted by Stephen New, Syngenta. Double-strand and four-fold sequencing coverage is provided for the regions of interest. All ambiguities were resolved on each strand. The consensus sequences match the Sponsor-provided reference sequences.

II. Amendments

This Report is amended as follows:

Amendment	Page	Reason
"Double-strand and four-fold sequencing coverage is provided for the regions of interest." is change to "Double-strand and four-fold sequencing coverage is provided for the regions of interest."	2	To correct grammar.
"Sequencing and PCR Primers" is changed to "Sequencing Primers"	4	Only Sequencing Primers were utilized.
"retained by GENWIZ" is changed to "retained by GENEWIZ"	5	To correct spelling.
"Additional applicable, cGMP measures is changed to: "Additional applicable cGMP measures"	7	To correct grammar.
"DNA Star" is changed to "DNASTAR"	4	DNASTAR is the "correct" spelling of the software name.
The GLP compliance statement is changed from: "AS PRINCIPAL INVESTIGATOR, I MAINTAIN THAT THIS PHASE WAS PERFORMED IN COMPLIANCE WITH APPLICABLE GOOD LABORATORY PRACTICE (GLP) REGULATION AND ANY NONCOMPLIANCE OR DEVIATIONS ARE ADDRESSED IN THIS REPORT." To: "AS PRINCIPAL INVESTIGATOR, I MAINTAIN THAT THIS PHASE WAS CONDUCTED IN COMPLIANCE WITH THE RELEVANT PROVISIONS OF GOOD LABORATORY PRACTICE STANDARDS (40 CFR PART 160) PURSUANT TO THE FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA). THIS PHASE PLAN WAS ALSO PERFORMED TO APPLICABLE GLP REGULATION AS PROMULGATED BY FDA 21CFR PART 58. ADDITIONAL APPLICABLE CGMP MEASURES HAVE ALSO BEEN APPLIED TO THIS PHASE. ANY NONCOMPLIANCE OR DEVIATIONS ARE ADDRESSED IN THIS REPORT."	7	The GLP Compliance Statement must indicate the specific GLP guidelines followed during the analytical phase (e.g. 40 CFR Part 160).
The QA statement is changed from "THIS PHASE REPORT IS COMPLETE AND ACCURATELY REFLECTS THE CONDUCT AND DATA OF THE PHASE PLAN. THIS PHASE PLAN WAS PERFORMED TO APPLICABLE GLP REGULATION AS PROMULGATED BY FDA 21CFR PART 58 AND US EPA GLPS, 40 CFR PART 160. ADDITIONAL APPLICABLE CGMP MEASURES HAVE ALSO BEEN APPLIED TO THIS PHASE. ALL QA AUDIT OBSERVATIONS HAVE BEEN SATISFACTORILY RESOLVED." To: "THIS STATEMENT VERIFIES THAT THE AFOREMENTIONED STUDY COMPLETE AND ACCURATELY REFLECTS THE CONDUCT AND DATA OF THE PHASE PLAN AND, PURSUANT TO GOOD	7	To increase accuracy of QA statement.

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

LABORATORY PRACTICE REGULATIONS (40 CFR PART 160 and 21 CFR PART 58), WAS INSPECTED AND/OR AUDITED AND THE FINDINGS REPORTED TO MANAGEMENT AND TO THE STUDY DIRECTOR BY THE QUALITY ASSURANCE UNIT ON THE DATES LISTED BELOW. ALL QA AUDIT OBSERVATIONS HAVE BEEN SATISFACTORILY RESOLVED."		
"Study Audit Report was sent to the Study Director" Section added to the QA Statement	7	Date must be included in Report

III. Phase Dates

Initiation Date: Sept. 1, 2011

Draft Report Date: Oct. 4, 2011

Completion Date: Oct. 14, 2011

Amendment Date: May 3, 2012

IV. Phase Results

The sequences of the regions of interest from the samples submitted by the Sponsor on 08/24/2011 were determined by the sequencing reactions described in this report. The consensus sequences match the Sponsor-provided reference sequences. The consensus sequences are shown in Attachment A.

V. Key GENEWIZ Project Personnel

Principal Investigator: [redacted] Date 3 May 2012

Associate Manager
Regulatory Services

Technical Scientist: [redacted] Date 4 May 2012

Associate Scientist II
Regulatory Services

Quality Assurance: [redacted] Date 4 May 2012

Director of Quality Assurance

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

VI. Test Article

The following test articles were provided by the Sponsor:

Test Article	Lot# (or Description)
DNA Fragments	A3-1, A3-2, A3-3; B4-1, B4-2, B4-3 C6-1, C6-2, C6-3; D8-1, D8-2, D8-3 E10-2, E10-3, E10-4; F11-1, F11-2, F11-3 G12-1, G12-2, G12-3; H17-1, H17-2, H17-3 I18-1, I18-2, I18-3; Vials of frozen stock received on 08/24/10

VII. Experimental Design and Methods Used

1. Test Article

Upon the initiation of this project, the project identifier "GW-Syn-001" was assigned. The sample was stored at -20 °C in freezer GW-0041.

The submitted frozen sample stock was thawed at room temperature. An aliquot was used for sequence reactions. The rest of the sample stock was kept at -20 °C in freezer GW-0041.

2. Sequencing Primers

The sequencing primers, which are listed in Attachment B, were designed by the Sponsor and GENEWIZ and synthesized by IDT. Primers are based on the reference sequence provided by the Sponsor.

3. Sequencing Reactions

Primer extension sequencing reactions were assembled using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA Polymerase, FS (Version 3.1, Applied BioSystems). GENEWIZ alternative protocols for the difficult regions were also used.

4. Sequencing Data Analysis

Sequencing reactions were analyzed on the ABI 3730xl automated DNA sequencer (Applied Biosystems) validated at GENEWIZ for GLP studies. Raw data was collected using data collection software version 3.0 (Applied BioSystems). The data was analyzed using Sequence Analysis software, Version 5.3 (Applied BioSystems). Sequence data was further analyzed and assembled using LaserGene Version 8 (DNASTAR).

5. Consensus Sequence Analysis

Each consensus sequence is derived from combining the sequence of three independent clones per fragments.

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

VIII. Acceptance Criteria

1. Raw data with quality scores (QS) of 40 and above.
2. Four-fold coverage for the region indicated.
3. Plasmid (pGEM) that is used as the reaction and instrument controls for each sequence reaction plate with QS ≥ 40 and a CRL (continuous read length) ≥ 800.
4. Control plasmid (pGEM) with two correct sequencing motifs, nt 24-27 (GGGG) and nt 479-487 (CCGTAAAAA), to control for plate orientation and to serve as identity QC markers.

IX. Record Retention

The phase records are retained as per SOP GW.018. Original signed reports are remitted to the Sponsor at the completion of the phase as per SOP GW.042.

GENEWIZ will transfer the original phase plan, original phase report, and all raw data and records, except for the facility-specific records, to Syngenta Crop Protection, LLC. for archiving within 90 days of study completion. Facility-specific records supporting this study will be retained by GENEWIZ for an indefinite period.

X. Sample Handling and Retention

The samples were tracked and retained as per SOP GW.007 and the Phase Plan. Samples will be stored at GENEWIZ for three months after the data is uploaded. After three months, samples will be discarded or returned to the Sponsor upon request.

XI. Delivery of Results

The project deliverables include the following items:

1. The final Phase Report reviewed and approved by GENEWIZ QA Unit.
2. GLP Statement provided by the Principal Investigator.
3. A signed QA Statement confirming that all work is completed in accordance with Good Laboratory Practice Standards (GLPS) as specified by 21 CFR, Part 58 and US EPA GLPS, 40 CFR Part 160.
4. A CD containing this Phase Report and the raw data in .ab1, .phd, and .seq formats.

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

XII. SOP Used in the Phase

SOP No.	Title
GW.006	Laboratory Notebook Issuance and Use
GW.007	Sample Handling and Tracking Procedure for Regulated Services
GW.231	Primer Design and Synthesis
GW.232	Single- and Double-Stranded Primer Walking
GW.129	Measuring OD of dsDNA using the NanoDrop Spectrophotometer
GW.125	Analysis, Quality Control Check, and Scoring of Sequencing Data
GW.115	DNA Sequencing Using Big Dye v3.1 Chemistry
GW.116	Alternative Protocols for Difficult Templates
GW.143	Post-Cycling Processing of Sequencing Plates Using MicroFlo Liquid Dispensers
GW.127	Plate Processing using Precipitation Protocol
GW.008	Installation, Calibration and Maintenance of the ABI 3730 DNA Analyzer
GW.009	Sequencing Data Back Up for ABI 3730
GW.011	Procedure for the Operation, Calibration, and Maintenance of Pipettes
GW.010	Installation, Calibration and Maintenance of the Thermocyclers
GW.015	Investigation of Out of Specification (OOS) and Unexpected results
GW.016	Change Control Procedure
GW.018	Document Control
GW.020	Procedure for Handling Deviations in GLP Studies
GW.026	Conducting and Monitoring of GLP Studies
GW.042	GLP Study Protocol & Report
GW.012	Preparing, Performing and Archiving a Study Audit by the QAU
GW.013	Preparing, Performing and Archiving a Facility Audit by the QAU

XIII. QA Oversight

Independent QA oversight of this phase was performed as promulgated by applicable 21CFR Part 58 and US EPA GLPS, 40 CFR Part 160 regulations. QA oversight of this phase included but was not limited to handling of the Master Schedule, Phase Protocol Approval, In-Process Auditing, Review and Approval of the Final Report and Archiving of phase documentation. A signed QA statement is included in this project report. Additional applicable cGMP measures have also been applied to this phase.

XIV. Table of Attachments

ATTACHMENT	PROCESS/TOPIC	# PAGES	ENTERED BY (DATE)
Attachment A	Consensus Sequences	5	Jeffrey Shaman (10/04/2011)
Attachment B	Sequencing Primers	1	Jeffrey Shaman (10/04/2011)
Attachment C	Full Length Contig	9	Jeffrey Shaman (10/04/2011)
Attachment D	Full Length Alignments (Reference vs Consensus)	16	Jeffrey Shaman (10/04/2011)

GENEWIZ®
Solid science. Superior service.

GENEWIZ, Inc.
115 Corporate Boulevard
South Plainfield, NJ 07080
p (877) GENEWIZ (436-3949)
f (908) 333-4511
www.genewiz.com

GLP COMPLIANCE STATEMENT

AS PRINCIPAL INVESTIGATOR, I MAINTAIN THAT THIS PHASE WAS CONDUCTED IN COMPLIANCE WITH THE RELEVANT PROVISIONS OF GOOD LABORATORY PRACTICE STANDARDS (40 CFR PART 160) PURSUANT TO THE FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT (FIFRA). THIS PHASE PLAN WAS ALSO PERFORMED TO APPLICABLE GLP REGULATION AS PROMULGATED BY FDA 21CFR PART 58. ADDITIONAL APPLICABLE CGMP MEASURES HAVE ALSO BEEN APPLIED TO THIS PHASE. ANY NONCOMPLIANCE OR DEVIATIONS ARE ADDRESSED IN THIS REPORT.

GLP Principal Investigator Signature/Date: [redacted] 3 May 2012

QA STAT[redacted]

THIS STATEMENT VERIFIES THAT THE AFOREMENTIONED STUDY COMPLETE AND ACCURATELY REFLECTS THE CONDUCT AND DATA OF THE PHASE PLAN AND, PURSUANT TO GOOD LABORATORY PRACTICE REGULATIONS (40 CFR PART 160 and 21 CFR PART 58), WAS INSPECTED AND/OR AUDITED AND THE FINDINGS REPORTED TO MANAGEMENT AND TO THE STUDY DIRECTOR BY THE QUALITY ASSURANCE UNIT ON THE DATES LISTED BELOW. ALL QA AUDIT OBSERVATIONS HAVE BEEN SATISFACTORILY RESOLVED.

QA Audits Performed

Phase/Stage	Date Conducted
In Process Audit	Oct 4, 2011
Draft Report & Raw Data No significant findings were found.	Oct. 14, 2011
Study Audit Report was sent to the Study Director	Feb. 1, 2012

QA Director Signature/Date: [redacted] / 4 May 2012

PHASE REPORT FINAL APPROVAL

Company Representative	Signature	Date
Principal Investigator	[redacted]	3 MAY 2012
QA		4 May 2012

APPENDIX A Sequencing Phase Report (Continued)

{Volume 2: CBI Cross-reference Number 3}

APPENDIX B SYHT0H2 Insert Sequence In Genbank Format

{Volume 2: CBI Cross-reference Number 4}