# SUMMARY

(In accordance with 40 CFR part 152, this summary is available for public release after registration)

## STUDY TITLE

Molecular Characterization of DAS-44406-6 Soybean

## DATA REQUIREMENTS

N/A

## AUTHOR(S)

Josh Poorbaugh

## STUDY COMPLETED ON

May 26, 2011

## PERFORMING LABORATORY

Regulatory Sciences and Government Affairs—Indianapolis Lab
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9330 Zionsville Road
Indianapolis, Indiana 46268-1054

## LABORATORY STUDY ID

101947

# Molecular Characterization of DAS-44406-6 Soybean

## SUMMARY

Soybean (*Glycine max* L.) event DAS-44406-6 was generated by *Agrobacterium*-mediated transformation of the variety "Maverick" using plasmid pDAB8264, followed by conventional breeding. Plasmid pDAB8264 contained a T-DNA insert with the following three plant transcription units (PTUs): 1) Histone H4A748 promoter, *2mEPSPS* gene, and Histone H4A748 3' UTR, 2) AtUbi10 promoter, *aad*-12 gene, and AtuORF23 3' UTR, 3) CsVMV promoter, *pat* gene, and AtuORF1 3' UTR. In addition, a RB7 matrix attachment region (RB7 MAR) is located at the 5' end of the T-DNA insert.

Molecular characterization of soybean event DAS-44406-6 was conducted by Southern blot analysis of individual plants across five generations using multiple enzyme digestions in combination with probes derived from the genetic elements in the transformation plasmid. Results demonstrated that a single T-DNA insert containing each of the intact PTUs for the *2mEPSPS*, *aad*-12 and *pat* genes were integrated into DAS-44406-6 soybean and the inserted DNA was stably inherited across the five generations evaluated. Moreover, the absence of transformation plasmid backbone sequence was demonstrated by probes covering nearly the entire region of the plasmid flanking the T-DNA insert.

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DATA REQUIREMENTS

N/A

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STUDY COMPLETED ON

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PERFORMING LABORATORY

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101947

# STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: AAD-12, PAT, 2mEPSPS

Title: Molecular Characterization of DAS-44406-6 Soybean

- STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS:

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Company: Dow AgroSciences LLC

Company Agent: M. Krieger

Title: Regulatory Manager

Signature: 

Date: 15 March 2011

THIS DATA MAY BE CONSIDERED CONFIDENTIAL IN COUNTRIES OUTSIDE THE UNITED STATES.

# STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Molecular Characterization of DAS-44406-6 Soybean

Study Initiation Date: 30-NOV-2010

This report represents data generated after the effective date of the EPA FIFRA Good Laboratory Practice Standards.

United States Environmental Protection Agency
Title 40 Code of Federal Regulations Part 160
FEDERAL REGISTER, August 17, 1989

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160.135 (b) with the following exceptions: The preparation of plasmid DNA used in the positive control samples and the generation of template DNA for probes were conducted in a non-GLP laboratory; The GLP status of the commercial reference standards and the primers for probe generation are unknown; The test substance was characterized in but not prior to this study.

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Sponsor
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Study Completion Date

**Dow AgroSciences Quality Assurance Unit**
**Good Laboratory Practice Statement Page**

**Study ID:** 101947

**Title:** Molecular Characterization of DAS-44406-06 Soybean

**Study Initiation Date:** 9/30/2010 **Study Completion Date:** 26-May-2011

**GLP Quality Assurance Inspections**

| Date of GLP Inspection(s) | Date Reported to the Study Director and to Management | Phases of the Study which received a GLP Inspection by the Quality Assurance Unit |
|---|---|---|
| 29-Nov-2010 | 29-Nov-2010 | Protocol Review |
| 13-Jan-2011 | 13-Jan-2011 | Membrane Treatment and Detection |
| 17, 18, 20-May-2011 | 20-May-2011 | Report and Raw Data Review |

**QUALITY ASSURANCE STATEMENT:**

The Quality Assurance Unit has reviewed the final study report and has determined that the report reflects the raw data generated during the conduct of this study.

Tfroggatt

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Dow AgroSciences, Quality Assurance

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Date

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Title: Molecular Characterization of DAS-44406-6 Soybean

Principal Analyst: Josh Poorbaugh
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# Molecular Characterization of DAS-44406-6 Soybean

## ABSTRACT

Soybean (*Glycine max* L.) event DAS-44406-6 was generated by *Agrobacterium*-mediated transformation of the variety "Maverick" using plasmid pDAB8264, followed by conventional breeding. Plasmid pDAB8264 contained a T-DNA insert with the following three plant transcription units (PTUs): 1) Histone H4A748 promoter, *2mEPSPS* gene, and Histone H4A748 3' UTR, 2) AtUbi10 promoter, *aad*-12 gene, and AtuORF23 3' UTR, 3) CsVMV promoter, *pat* gene, and AtuORF1 3' UTR. In addition, a RB7 matrix attachment region (RB7 MAR) is located at the 5' end of the T-DNA insert.

Molecular characterization of soybean event DAS-44406-6 was conducted by Southern blot analysis of individual plants across five generations using multiple enzyme digestions in combination with probes derived from the genetic elements in the transformation plasmid. Results demonstrated that a single T-DNA insert containing each of the intact PTUs for the *2mEPSPS, aad*-12 and *pat* genes were integrated into DAS-44406-6 soybean and the inserted DNA was stably inherited across the five generations evaluated. Moreover, the absence of transformation plasmid backbone sequence was demonstrated by probes covering nearly the entire region of the plasmid flanking the T-DNA insert.

# ABBREVIATIONS

| | |
|---|---|
| 2mEPSPS | Double mutated 5-EnolPyruvylShikimate-3-Phosphate Synthase |
| AAD-12 | AryloxyAlkanoate Dioxygenase-12 |
| AP | Alkaline Phosphatase |
| bp | Base pair |
| CTAB | CetylTrimethylAmmonium Bromide |
| °C | Degrees Celsius |
| DNA | Deoxyribonucleic acid |
| DIG | Digoxigenin |
| dUTP | Deoxyuridine triphosphate |
| EDTA | Ethylenediaminetetraacetic acid |
| kb | Kilobase |
| LFS | Lateral Flow Strip |
| µg | Microgram |
| µL | Microliter |
| mL | Milliliter |
| M | Molar mass |
| PAT | Phosphinothricin acetyl transferase |
| PCR | Polymerase chain reaction |
| PTU | Plant transcription unit |
| SDS | Sodium dodecyl sulfate |
| SSC | Buffer solution containing a mixture of sodium chloride and sodium citrate |
| TBE | Buffer solution containing a mixture of Tris base, boric acid and EDTA |

# INTRODUCTION

Soybean (*Glycine max* L.) event DAS-44406-6 was generated by *Agrobacterium*-mediated transformation of the variety "Maverick" using plasmid pDAB8264, followed by conventional breeding. Plasmid pDAB8264 contains a T-DNA insert with the following three plant transcription units (PTUs): 1) Histone H4A748 promoter, *2mEPSPS* gene and Histone H4A748 3' UTR, 2) AtUbi10 promoter, *aad*-12 gene and AtuORF23 3' UTR, 3) CsVMV promoter, *pat* gene and AtuORF1 3' UTR. In addition, a RB7 matrix attachment region (RB7 MAR) is located at the 5' end of the T-DNA insert.

The purpose of this study was to characterize the transgenic insert in soybean event DAS-44406-6. Southern blot analysis was used to determine the integration pattern of the inserted DNA fragment and the insert number of the *2mEPSPS*, *aad*-12 and *pat* PTUs. Data were generated to evaluate the integration and integrity of the *2mEPSPS*, *aad*-12 and *pat* genes inserted into the soybean genome. Characterization of the integration of non-coding regions (designed to regulate the coding regions), such as promoters, terminators and RB7 MAR, were also performed. Stability of the T-DNA insert across five generations was investigated. Moreover, probes covering nearly the entire backbone of pDAB8264 were used to demonstrate absence of plasmid backbone sequence in the DAS-44406-6 soybean.

# MATERIALS AND METHODS

## Test Substance/Test System

The test substance in this study was the DAS-44406-6 soybean event. Genomic DNA was extracted from leaf tissue harvested from individual plants of five generations of DAS-44406-6 soybean for evaluation of the genetic elements. Seeds from each generation of DAS-44406-6 soybean, along with the source identification, were obtained from the Trait Product Development group in Dow AgroSciences (Guttikonda) (Table 1). The genomic DNA from the transgenic and non-transgenic soybeans were the test systems in this study.

## Control Substances

The control substance used in this study was the unmodified soybean variety Maverick. The unmodified plants have a genetic background representative of the test substance lines, but do not contain genes for the 2mEPSPS, PAT or AAD-12 proteins. The conventional soybean seeds were provided by the Trait Product Development in Dow AgroSciences (Guttikonda) (Table 1).

## Reference Materials

The 1 kb plus DNA ladder (Invitrogen) and the DIG DNA Markers II and VII (Roche Diagnostics) were used for agarose gel electrophoresis and/or Southern blot analysis. Each marker contains a mixture of DNA fragments with different sizes to serve as size reference material.

Plasmid pDAB8264 was used for *Agrobacterium*-transformation to generate event DAS-44406-6. This plasmid, therefore, serves as a positive control for the *2mEPSPS*, *pat* and *aad*-12 transgenes, as well as the other genetic features evaluated in event DAS-44406-6. The reference plasmid was added to DNA samples from conventional soybean plants (Maverick) for Southern blot analysis at approximately one copy per genome (~72pg).

Seed Planting, Primary Characterization and Leaf Harvest

Soybean seeds from five generations of DAS-44406-6, as well as the non transgenic variety "Maverick" were planted in an Indianapolis greenhouse and harvested as described in Guttikonda *et al* (Guttikonda). Briefly, at least five seeds from each generation were planted and emerged plants were evaluated for PAT protein expression by lateral flow strip (LFS) tests (Envirologix). The leaf tissue of positive-expressing transgenic plants, as well as the Maverick, were then harvested and quickly frozen in liquid nitrogen and stored at approximately -80°C until usage.

Genomic DNA Extraction

Individual genomic DNA was extracted from the frozen soybean leaf tissue following a modified CTAB (CetylTrimethylAmmonium Bromide) method. Briefly, each leaf sample was ground in liquid nitrogen followed by the addition of ~25 mL CTAB extraction buffer (1% CTAB, 1.4M NaCl, 50 mM Tris-HCl, 10 mM EDTA), RNase-A (~50 µL) and Proteinase K (~50 µL). After approximately 1-2 hours of incubation at ~65 °C with gentle shaking, samples were spun down and the supernatants were extracted with an equal volume of chloroform : octanol = 24:1, twice. DNA was then precipitated by mixing the supernatants with equal volume of CTAB precipitation buffer (1% CTAB, 50 mM Tris-HCl, 10 mM EDTA). The precipitated DNA was dissolved in high salt TE buffer (1× TE pH8.0, 1.0M NaCl) followed by precipitation with isopropyl alcohol. The precipitated DNA was rinsed with 70% ethanol, air-dried and then dissolved in appropriate volume of 1× TE buffer (pH8.0). To check the quality of the resultant genomic DNA, an aliquot of the DNA samples was electrophoretically separated on a 1% agarose gel containing ethidium bromide (~1 µg/mL) with 1× TBE buffer. The gel was visualized under ultraviolet (UV) light to confirm that the DNA was not degraded and that the RNA had been removed by the RNase-A. The concentration of DNA in solution was determined by the PicoGreen Kit (Invitrogen) in a fluorometer (Bio-TEK, Model #:FLX800).

DNA Probes

DNA probes specific to the *2mEPSPS*, *pat*, and *aad*-12 genes and the other elements in the plasmid pDAB8264 were produced by polymerase chain reaction (PCR) amplification using pDAB8264 plasmid DNA (Figure 1) as template. A list of probes used for the study is described in Table 2.

DNA Digestion and Separation

For molecular characterization of the DNA, ten micrograms (10µg) of genomic DNA from event DAS-44406-6 and the conventional control were digested by adding approximately 5-10 units of selected restriction enzyme per µg of DNA and the corresponding reaction buffer to each DNA sample. Each sample was incubated at approximately 37 °C overnight. The restriction enzymes *Msc*I, *Xho*I, *Pst*I/*Xho*I, *Msc*I/*Eco*RI, and *Hin*dIII (New England Biolabs) were used singly, in combination or sequentially for the digestions. The positive hybridization control sample was prepared by combining plasmid DNA pDAB8264 with genomic DNA from the conventional control at a ratio of approximately equivalent to one copy of transgene per soybean genome (~72 pg), and digested using the same procedures and restriction enzyme(s) as the test samples. DNA from the conventional soybean control (Maverick) was digested using the same procedures and restriction enzymes as the test samples to serve as a negative control.

The digested DNA samples were precipitated with Quick-Precip reagent (Edge BioSystems) and resuspended in 1× Loading Dye (Invitrogen) to achieve the desired volume for gel loading. The DNA samples and molecular size markers were then electrophoresed through 0.8% agarose gels with 1×TBE buffer at 55-65 volts overnight to achieve fragment separation. The gels were stained with ethidium bromide and the DNA was visualized under ultraviolet (UV) light. A photographic record was made for each stained gel.

Southern Transfer and Membrane Treatment

Southern blot analysis was performed essentially as described by Dow Agrosciences SOPs and Memelink, et al (Memelink *et al.*). Briefly, following electrophoretic separation and

visualization of the DNA fragments, the gels were depurinated by 0.25N HCl for ~20 minutes and then exposed to a denaturing solution (1.5 M NaCl, 0.5 N NaOH) for ~30 minutes followed by a neutralizing solution (1.5 M NaCl, 1.0 M Tris-HCl, pH 7.5) for at least ~30 minutes. Southern transfer was performed onto nylon membranes overnight using a wicking system with 10× SSC. After transfer, the membranes were washed in a 2× SSC solution and the DNA was fixed to the membrane by UV crosslinking. This process resulted in Southern blot membranes ready for hybridization.

DNA Probe Labeling and Hybridization

The DNA fragments bound to the nylon membrane were detected using a labeled probe. Probes used for the study were generated by a PCR-based incorporation of a digoxigenin (DIG) labeled nucleotide, [DIG]-dUTP, into fragments generated by primers specific to gene elements and other regions from plasmid pDAB8264. Generation of DNA probes by PCR synthesis was carried out using a PCR DIG Probe Synthesis Kit (Roche Diagnostics).

Labeled probes were analyzed by agarose gel electrophoresis and PicoGreen to determine their quality and quantity, respectively. A desired amount of labeled probe was then used for hybridization to the target DNA on the nylon membranes for detection of the specific fragments essentially as described in the manufacturer's procedures (Roche). Briefly, nylon membrane blots with DNA fixed on were briefly washed with 2×SSC and prehybridized with 20-25 mL of prewarmed DIG Easy Hyb solution in hybridization bottles at approximately 40-68°C for a minimum of 30 minutes in a hybridization oven. The prehybridization solution was then decanted and replaced with ~20 mL of prewarmed DIG Easy Hyb solution containing a desired amount of specific probes previously denatured by boiling in a water bath for approximately five minutes. The hybridization step was then conducted at approximately 55-65°C overnight in the hybridization oven.

Detection

At the end of the probe hybridization, DIG Easy Hyb solutions containing the probes were decanted into clean tubes and stored at approximately -20°C. The membrane blots were rinsed briefly and washed twice with low stringency wash buffer (2×SSC, 0.1%SDS) for approximately five minutes at room temperature, followed by washing twice with high stringency wash buffer (0.1×SSC, 0.1% SDS) for 15 minutes each, at approximately 65°C. This was followed by blocking in a 1× blocking buffer for a minimum of 30 minutes and incubation with anti-DIG-AP (Alkaline Phosphatase) antibody (Roche Diagnostics, 1:5,000 dilution) in 1× blocking buffer also for a minimum of 30 minutes. After three washes with 1× washing buffer, specific DNA probes that remained bound to the membrane blots and DIG-labeled DNA standards were visualized using CDP-Star Chemiluminescent Nucleic Acid Detection System (Roche Diagnostics) following the manufacturer's recommendation. Blots were exposed to chemiluminescent film (Roche Diagnostics) for one or more time points to detect hybridizing fragments and to visualize molecular size standards. Films were developed with an All-Pro 100 Plus film developer (Konica Model #: SRX-101) and images were scanned for report. DIG-labeled DNA Molecular Weight Marker II and VII (DIG MWM II and DIG MWM VII), visible after DIG detection as described, were used to determine hybridizing fragment size on the Southern blots.

Probe Stripping

DNA probes were stripped from the membrane blots after the Southern hybridization data were obtained, and the membrane blots could be reused for hybridization with a different DNA probe according to the manufacturer's recommended procedures (Roche). Briefly, after signal detection and film exposure, membrane blots were thoroughly rinsed with Milli-Q water and then washed twice in stripping buffer (0.2N NaOH, 0.1% SDS) for approximately 15 minutes at room temperature. The membrane blots were then briefly washed in 2×SSC and were ready for prehybridization and hybridization with another DNA probe.

## RESULTS AND DISCUSSION

Genomic DNA samples extracted from individual plants from five distinct generations (three plants per generation) of soybean event DAS-44406-6 were selected for molecular characterization (Table 1). Expected and observed fragment sizes with a particular digestion and probe combination, based on the expected restriction enzyme sites in plasmid pDAB8264 (Figure 1) and the hypothesized linearized T-DNA insert (Figure 2), are given in Table 3.

### Number of Insertion Sites

The restriction endonucleases *Msc*I, *Xho*I and *Hin*dIII were chosen to determine the number of insertions in the DAS-44406-6 soybean (Figure 1 and Figure 2). Probes derived from the DNA sequences for *2mEPSPS*, Histone UTR, Histone Promoter, *aad*-12, *pat*, RB7, AtUbi10 Promoter, AtuORF23 UTR, CsVMV and AtuORF1 UTR were then hybridized to the samples to determine the number of insertion sites for the DAS-44406-6 soybean.

When digested with the *Msc*I restriction enzyme and independently probed with the Histone UTR, Histone Promoter, AtUbi10 Promoter, *2mEPSPS*, *aad*-12 and *pat* probes, a single band of ~15000 bp was observed in all DAS-44406-6 samples, agreeing with the predicted size of >9328 bp in Figure 2 (Figure 4B, Figure 5B, Figure 6B, Figure 10C, Figure 11C and Figure 12C respectively). The same enzyme digestion was also used for characterization of the RB7 MAR feature. The resulting Southern analysis indicated a single band of ~3400 bp in DAS-44406-6 samples correlating with the expected size of >1330 bp (Figure 3B).

For additional characterization of the T-DNA insert, the DNA was digested with the *Xho*I restriction enzyme and independently probed with the Histone Promoter, AtUbi10 Promoter, AtuORF23 UTR, CsVMV, AtuORF1 UTR, *2mEPSPS*, *aad*-12 and *pat* probes. In each case, a single band of ~12000 bp was observed in all DAS-44406-6 samples, agreeing with the predicted size of >10093 bp in Figure 2 (Figure 5A, Figure 6A, Figure 7A, Figure 8A, Figure 9A, Figure 10A, Figure 11A and Figure 12A respectively).

Lastly, the restriction enzyme *Hin*dIII was used to provide further characterization of DAS-44406-6 soybean. Digestion of the genomic DNA with this enzyme followed by independently probing with the RB7, Histone UTR or *2mEPSPS* probe resulted in an identical, single band of ~4700 bp across all DAS-44406-6 samples, which correlated with the expected size of >4261 bp from Figure 2 (Figure 3A, Figure 4A and Figure 10B respectively). Digestion of the genomic DNA with *Hin*dIII and probed independently with the AtuORF23 UTR, CsVMV, AtuORF1 UTR, *aad*-12 or *pat* probe resulted in a single band of ~7000 bp for all DAS-44406-6 samples. This hybridization pattern agreed with the expected size of >4432 bp for all probe combinations (Figure 7B, Figure 8B, Figure 9B, Figure 11B and Figure 12B respectively).

Structure of the Insert and Genetic Elements

According to the restriction map of the T-DNA insert in pDAB8264 in Figure 2, the plant transcription unit (PTU) for both *aad*-12 and *pat* could be released by restriction digestion with *Pst*I/*Xho*I, while the *2mEPSPS* PTU may be released with a combination of *Msc*I/*Eco*RI. These digestions were performed in order to verify the presence of intact PTUs in DAS-44406-6 soybean.

When digested with *Msc*I/*Eco*RI and hybridized with the *2mEPSPS* probe, each individual DAS-44406-6 plant across the five generations, along with the pDAB8264 positive control, displayed a single band around 4500 bp, which agreed with the predicted size of 4469 bp for the *2mEPSPS* PTU (Table 3, Figure 13). When the same genomic DNA samples were probed with the Histone Promoter or Histone UTR probe, the same band of ~4500 bp was detected (Figure 13). This data indicates that an intact *2mEPSPS* PTU is present in all generations of DAS-44406-6 soybean tested.

Analysis of the *aad*-12 PTU, excised by *Pst*I/*Xho*I and hybridized with an *aad*-12 probe, resulted in a single band of ~2900 bp, which agreed with the predicted size of 2868 for the *aad*-12 PTU in each of the five generations and the positive control, pDAB8264 (Table 3, Figure 14). When the same genomic DNA samples were stripped and analyzed again with probes for the AtUbi10 Promoter or AtuORF23 UTR, the same band of ~2900 bp was also detected (Figure 14). This data indicates that an intact *aad*-12 PTU is present in all generations of DAS-44406-6 soybean tested.

When digested with *Pst*I/*Xho*I and hybridized with *pat* probe, each individual plant across the five generations of DAS-44406-6 soybean along with the pDAB8264 positive control displayed a single band around 1900 bp, which agree with the predicted size of 1928 bp for the *pat* PTU (Table 3, Figure 15). When the same genomic DNA samples were probed with the CsVMV or AtuORF1 UTR probe, the same band of ~1900 bp was detected in the same set of samples only (Figure 15). This data indicates that an intact *pat* PTU is present in all generations tested.

As expected, no specific hybridization bands were detected in the negative control samples in any of the restriction enzyme and probe combinations.

Taken together, the Southern blot analysis indicates that the single insert in DAS-44406-6 soybean contains intact PTUs for *2mEPSPS*, *aad*-12 and *pat*.

Absence of Backbone Sequences

In order to verify that no plasmid vector backbone sequences were inserted in soybean event DAS-44406-6, six probes covering the region of pDAB8264 plasmid DNA outside of the T-DNA were generated and hybridized to blots from digestions of *Msc*I/*Eco*RI, *Hin*dIII and *Pst*I/*Xho*I (Table 2 and Figure 1). Based on the expected *Msc*I/*Eco*RI and *Hin*dIII digestion fragment sizes, the Backbone 3 and Backbone 4 probes were mixed at an approximate 1:1 molar ratio for hybridization purposes to verify lack of backbone insertion. The probes Ori-Rep,

Backbone 1, Backbone 2 and SpecR were hybridized independently on separate *Hin*dIII and *Pst*I/*Xho*I blots. The results demonstrated that no specific hybridization bands were detected in any samples tested, except for the positive controls, as expected (Figure 16, Figure 17, Figure 18, Figure 19 and Figure 20). For the positive control in the *Msc*I/*Eco*RI digestion, the Backbone 4 probe had an expected and observed size of ~5900 bp, while the Backbone 3 probe had an expected and observed size of ~1000 bp (Figure 16A). For the same two probes, when digested with *Hin*dIII, the positive control had an expected and observed size of ~9300 bp for the Backbone 4 probe, while the Backbone 3 probe had an expected and observed size of ~4700 bp (Figure 16B). The Ori-Rep, Backbone 1, Backbone 2 and SpecR probes displayed the expected single band at ~9300 bp for the *Hin*dIII digestion, while they demonstrated the expected band of ~9300 bp for the *Pst*I/*Xho*I digestion (Figure 17, Figure 18, Figure 19 and Figure 20 respectively). These data confirmed that no backbone sequences from pDAB8264 have been incorporated into soybean event DAS-44406-6.

## CONCLUSIONS

The Southern blot data in this study confirmed that soybean event DAS-44406-6 contains a single insertion of the T-DNA from plasmid pDAB8264 with intact PTUs for *2mEPSPS*, *aad*-12 and *pat*. Identical hybridization patterns were observed with all enzyme digestion and probe combinations from soybean plants across five distinct generations of event DAS-44406-6, indicating stability of inheritance across multiple generations. Moreover, the absence of the backbone sequences from plasmid pDAB8264 in DAS-44406-6 soybean was confirmed using probes covering nearly the entire regions flanking the T-DNA insert.

## ARCHIVING

The protocol, raw data, and the original version of the final report will be filed in the Dow AgroSciences LLC archives at 9330 Zionsville Road in Indianapolis, IN 46268-1054.

## STATISTICAL TREATMENT OF DATA

No statistical methods are used in this study.

# REFERENCES

Guttikonda, S., Cruse, J., 2011. Characterization of DAS-44406-6 Soybean by Lateral Flow Strip and Event-Specific PCR. Dow AgroSciences LLC, Indianapolis.

Memelink, J., Swords, M.M.K., Staehelin, L.A., Hoge, C.H.J., 1994. Southern, Northern, and Western Blot Analysis. Plant Molecular Biology Manual F1, 1-23.

Roche, 2000. DIG Application Manual for Filter Hybridization. Roche Molecular Biochemicals, Germany.

Table 1. Description of Soybean Generations Used for Genomic DNA Extraction

| Sample ID* | Generation | Source ID |
|---|---|---|
| 4406-F2-X | F2 | YX10BX026010.0007.001 |
| 4406-T2-X | T2 | YX09AX001736.110 |
| 4406-T3-X | T3 | YW09EW023005.0177.177 |
| 4406-T4-X | T4 | YX09KX590371.044 |
| 4406-T6-X | T6 | YT10ET450586.0005.005 |
| Maverick-X | Null | YW10EW000012.0001 |

* The -X following the Sample ID indicates plant number

Table 2. Description of DNA Probes Used for Southern Hybridization

| Gene/Probe | Position of pDAB8264 | Size (bp) |
|---|---|---|
| RB7 | 306-1315 | 1010 |
| Histone UTR | 1356-1907 | 552 |
| *2mEPSPS* | 2048-3759 | 1712 |
| Histone Promoter | 3682-5197 | 1516 |
| AtUbi10 Promoter | 5347-6659 | 1313 |
| *aad*-12 | 6616-7497 | 882 |
| AtuORF23 UTR | 7637-8049 | 413 |
| CsVMV | 8172-8703 | 532 |
| *pat* | 8676-9284 | 609 |
| AtuORF1 UTR | 9257-10055 | 799 |
| Backbone 3 | 10670-10990 | 321 |
| Ori-Rep | 10971-12057 | 1087 |
| Backbone 2 | 12038-13751 | 1714 |
| Backbone 1 | 13721-14974 | 1254 |
| SpecR | 14955-15749 | 795 |
| Backbone 4 | 15724-16015 | 292 |

Table 3. Expected and Observed Hybridization Fragments on Southern Blots with Gene Element Probes for Event DAS-44406-6

| Probe/ Feature | Enzyme | Sample | Expected Result (bp)[1] | Observed Result (bp)[2] | Figure # |
|---|---|---|---|---|---|
| RB7 | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 3A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4261 | ~4700 | |
| | *Msc*I | pDAB8264 | 5929 | ~5900 | 3B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >1330 | ~3400 | |
| Histone UTR | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 4A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4261 | ~4700 | |
| | *Msc*I | pDAB8264 | 10089 | ~10100 | 4B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >9328 | ~15000 | |
| Histone Promoter | *Xho*I | pDAB8264 | 16018 | ~16000 | 5A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Msc*I | pDAB8264 | 10089 | ~10100 | 5B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >9328 | ~15000 | |
| AtUbi10 Promoter | *Xho*I | pDAB8264 | 16018 | ~16000 | 6A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Msc*I | pDAB8264 | 10089 | ~10100 | 6B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >9328 | ~15000 | |
| AtuORF 23 UTR | *Xho*I | pDAB8264 | 16018 | ~16000 | 7A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Hin*dIII | pDAB8264 | 4731 | ~4700 | 7B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4432 | ~7000 | |
| CsVMV | *Xho*I | pDAB8264 | 16018 | ~16000 | 8A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Hin*dIII | pDAB8264 | 4731 | ~4700 | 8B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4432 | ~7000 | |
| AtuORF1 UTR | *Xho*I | pDAB8264 | 16018 | ~16000 | 9A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Hin*dIII | pDAB8264 | 4731 | ~4700 | 9B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4432 | ~7000 | |

Table 3 Cont. Expected and Observed Hybridization Fragments on Southern Blots with Gene Element Probes for Event DAS-44406-6

| Probe/ Feature | Enzyme | Sample | Expected Result (bp)[1] | Observed Result (bp)[2] | Figure # |
|---|---|---|---|---|---|
| *2mEPSPS* | *Xho*I | pDAB8264 | 16018 | ~16000 | 10A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 10B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4261 | ~4700 | |
| | *Msc*I | pDAB8264 | 10089 | ~10100 | 10C |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >9328 | ~15000 | |
| *aad*-12 | *Xho*I | pDAB8264 | 16018 | ~16000 | 11A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Hin*dIII | pDAB8264 | 4731 | ~4700 | 11B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4432 | ~7000 | |
| | *Msc*I | pDAB8264 | 10089 | ~10100 | 11C |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >9328 | ~15000 | |
| *pat* | *Xho*I | pDAB8264 | 16018 | ~16000 | 12A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >10093 | ~12000 | |
| | *Hin*dIII | pDAB8264 | 4731 | ~4700 | 12B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >4432 | ~7000 | |
| | *Msc*I | pDAB8264 | 10089 | ~10100 | 12C |
| | | Maverick | None | None | |
| | | DAS-44406-6 | >9328 | ~15000 | |

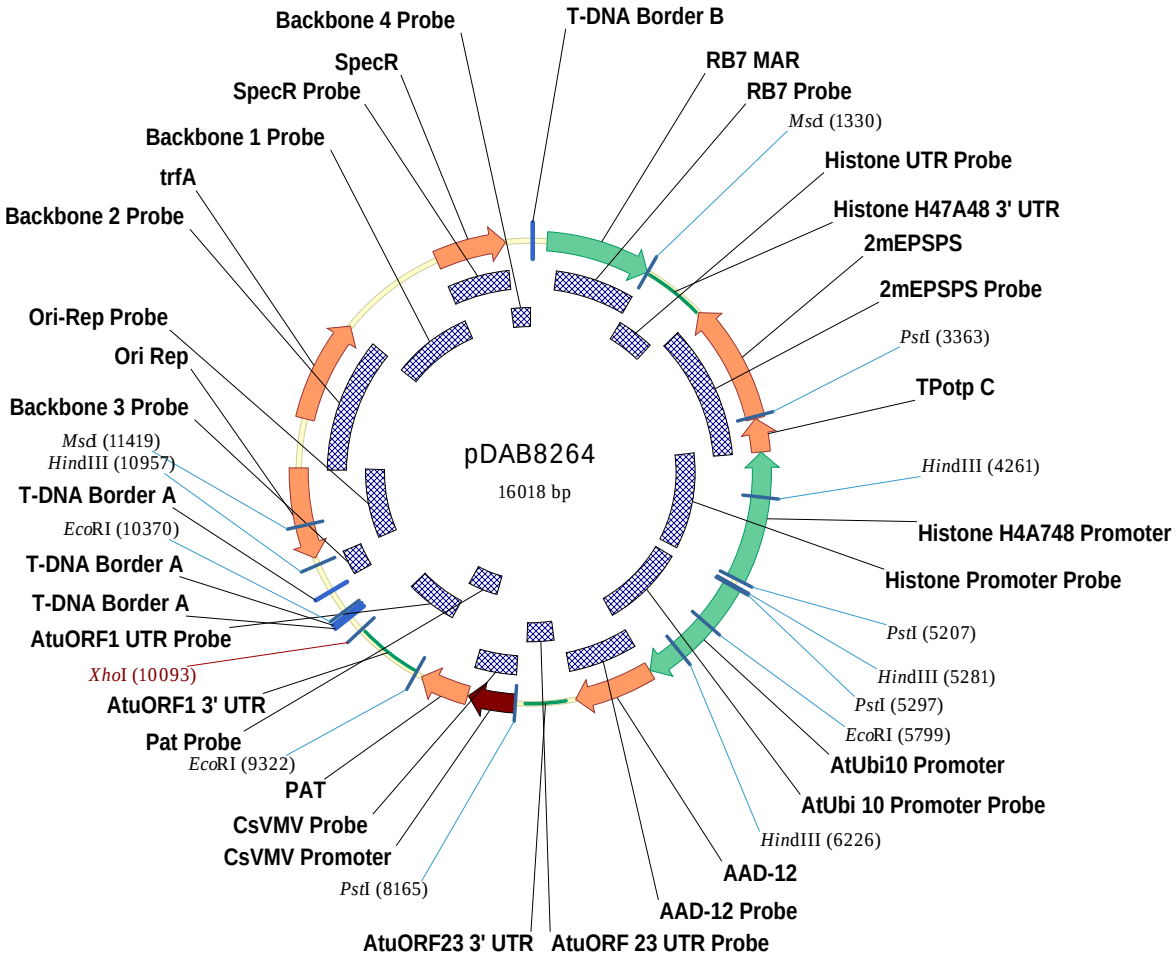
Table 3 Cont. Expected and Observed Hybridization Fragments on Southern Blots with Gene Element Probes for Event DAS-44406-6

| Probe/ Feature | Enzyme | Sample | Expected Result (bp)[1] | Observed Result (bp)[2] | Figure # |
|---|---|---|---|---|---|
| Histone Promoter | *Msc*I / *Eco*RI (Release PTU) | pDAB8264 | 4469 | ~4500 | 13A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 4469 | ~4500 | |
| *2mEPSPS* | | pDAB8264 | 4469 | ~4500 | 13B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 4469 | ~4500 | |
| Histone UTR | | pDAB8264 | 4469 | ~4500 | 13C |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 4469 | ~4500 | |
| AtUbi10 Promoter | *Pst*I / *Xho*I (Release PTU) | pDAB8264 | 2868 | ~2900 | 14A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 2868 | ~2900 | |
| *aad*-12 | | pDAB8264 | 2868 | ~2900 | 14B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 2868 | ~2900 | |
| AtuORF23 UTR | | pDAB8264 | 2868 | ~2900 | 14C |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 2868 | ~2900 | |
| CsVMV | *Pst*I / *Xho*I (Release PTU) | pDAB8264 | 1928 | ~1900 | 15A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 1928 | ~1900 | |
| *pat* | | pDAB8264 | 1928 | ~1900 | 15B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 1928 | ~1900 | |
| AtuORF1 UTR | | pDAB8264 | 1928 | ~1900 | 15C |
| | | Maverick | None | None | |
| | | DAS-44406-6 | 1928 | ~1900 | |

Table 3 Cont. Expected and Observed Hybridization Fragments on Southern Blots with Gene Element Probes for Event DAS-44406-6

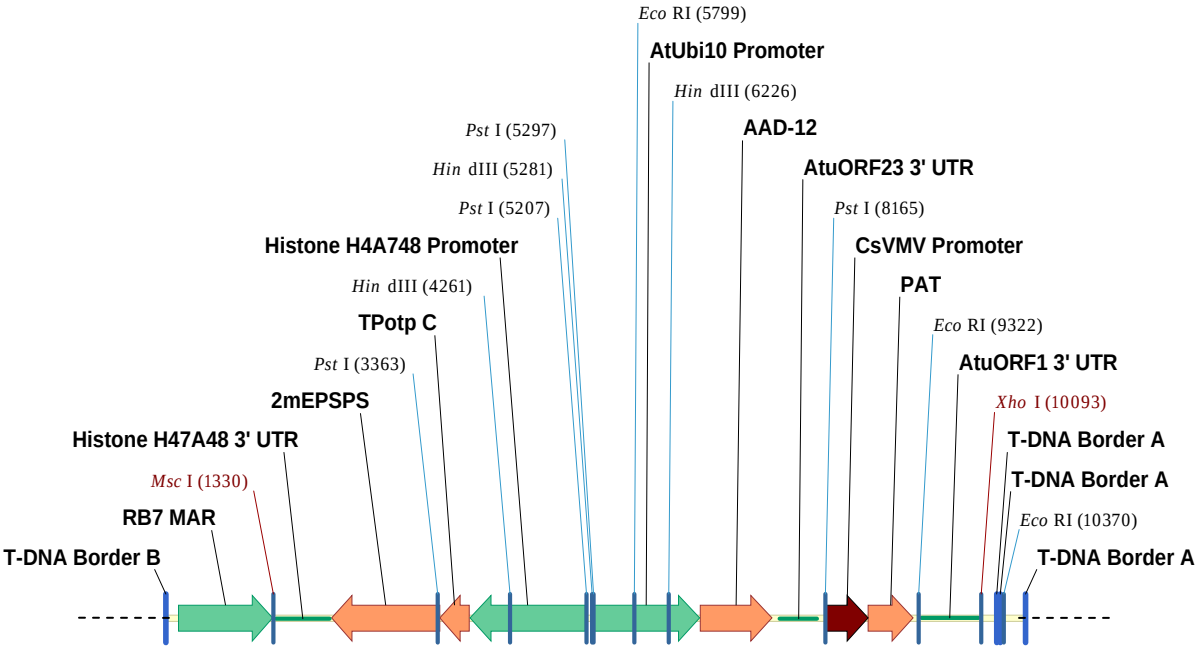
| Probe/ Feature | Enzyme | Sample | Expected Result (bp)[1] | Observed Result (bp)[2] | Figure # |
|---|---|---|---|---|---|
| Backbone 3 / Backbone 4 | *Msc*I / *Eco*RI | pDAB8264 | 1049, 5929 | ~1000, ~5900 | 16A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| | *Hin*dIII | pDAB8264 | 4731, 9322 | ~4700, ~9300 | 16B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| Ori-Rep | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 17A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| | *Pst*I / *Xho*I | pDAB8264 | 9288 | ~9300 | 17B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| Backbone 1 | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 18A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| | *Pst*I / *Xho*I | pDAB8264 | 9288 | ~9300 | 18B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| Backbone 2 | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 19A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| | *Pst*I / *Xho*I | pDAB8264 | 9288 | ~9300 | 19B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| SpecR | *Hin*dIII | pDAB8264 | 9322 | ~9300 | 20A |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |
| | *Pst*I / *Xho*I | pDAB8264 | 9288 | ~9300 | 20B |
| | | Maverick | None | None | |
| | | DAS-44406-6 | None | None | |

1. Expected fragment sizes are based on the plasmid map of the pDAB8264 (Figure 1) and the linearized T-DNA map (Figure 2).
2. Observed fragment sizes are considered approximately from these analyses and are based on the indicated sizes of the DIG-labeled DNA Molecular Weight Marker fragments. Due to the incorporation of DIG molecules for visualization, the Marker fragments typically run approximately 5-10% larger than their actual indicated molecular weight.




Plasmid pDAB8264 annotated with restriction enzyme sites as well as probes designed for specific elements to demonstrate coverage of the plasmid to be characterized.

Figure 1. Schematic Diagram of Plasmid pDAB8264



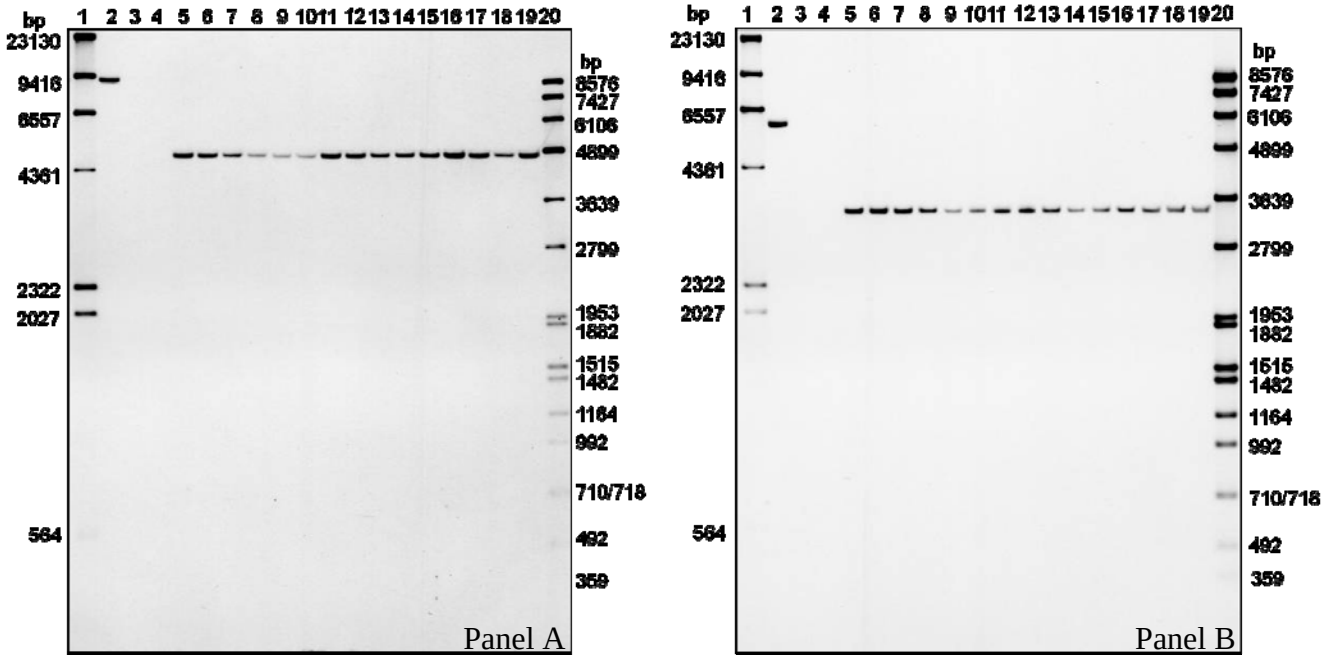

pDAB8264 Insert

10658 bp

| | | | | | |
|---|---|---|---|---|---|
| *Msc*I | >1330 | >9328 | | | |
| *Xho*I | >10093 | | | | |
| *Hin*dIII | >4261 | | | >4432 | |
| *Pst*I/*Xho*I | >3363 | 1844 | 2868 | 1928 | |
| *Msc*I/*Eco*RI | 4469 | 3523 | | | |

A 10658 bp T-DNA fragment from plasmid pDAB8264 is hypothesized to have inserted into the plant genome. Restriction enzyme sites are given with bp positions. Below the schematic are fragments expected to be observed on Southern blot hybridization experiments with restriction enzymes indicated.

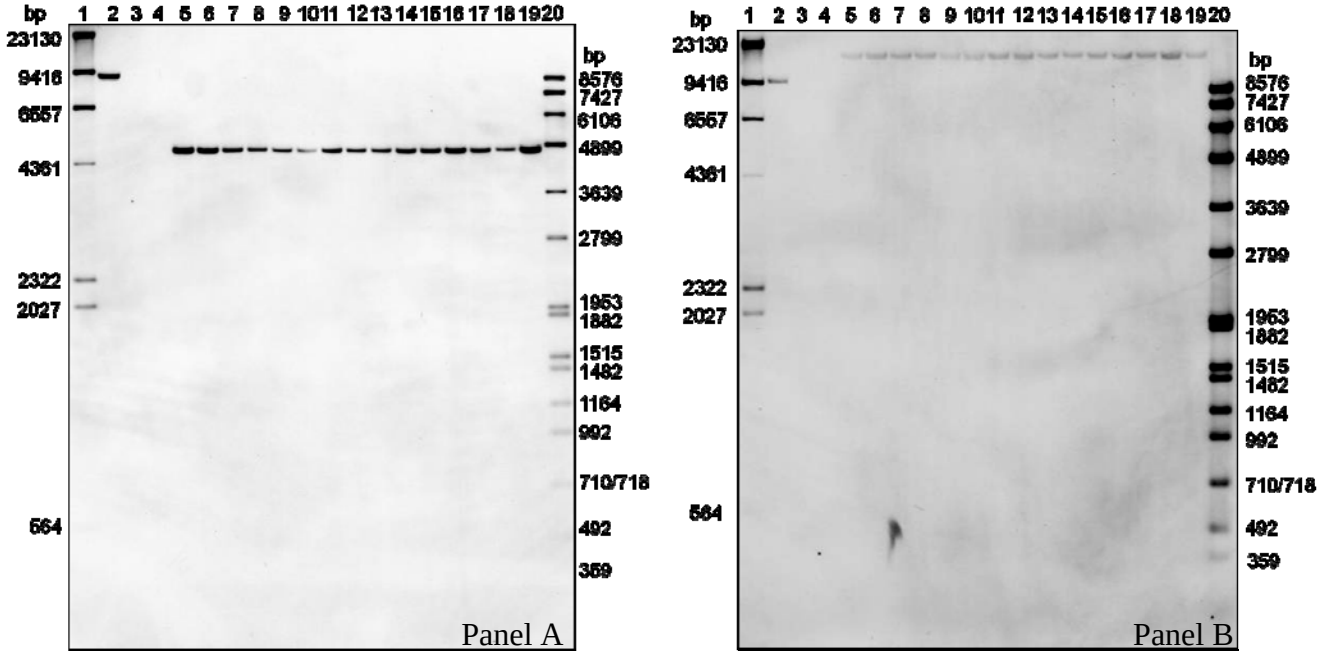
Figure 2. Map of the T-DNA Insert from pDAB8264 with Restriction Enzyme Sites




| Panel A | | | | Panel B | | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 μg of DAS-44406-6 genomic DNA was digested with *Hin*dIII (Panel A) and *Msc*I (Panel B) and hybridized with the RB7 probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

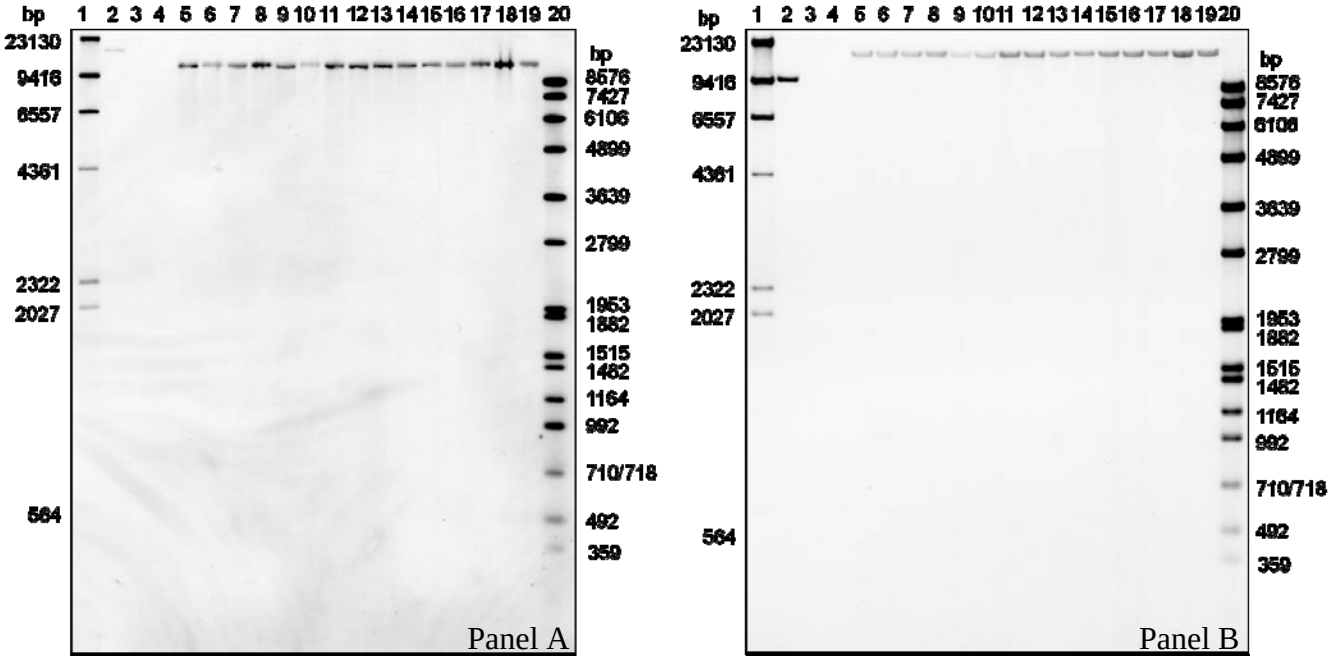
Figure 3. Southern Blot Analysis of DAS-44406-6 Digested with *Hin*dIII (Panel A) and *Msc*I (Panel B) and Detected with the RB7 Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Hin*dIII (Panel A) and *Msc*I (Panel B) and hybridized with the Histone UTR probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

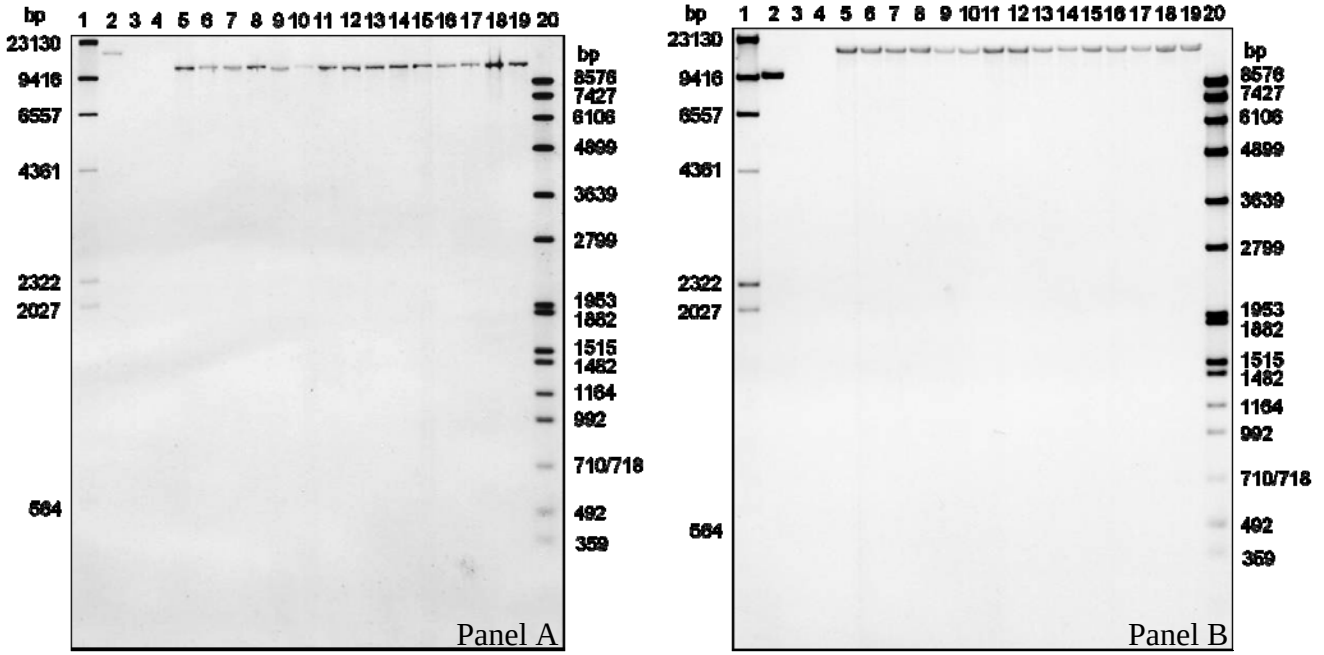
Figure 4. Southern Blot Analysis of DAS-44406-6 Digested with *Hin*dIII (Panel A) and *Msc*I (Panel B) and Detected with the Histone UTR Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A) and *Msc*I (Panel B) and hybridized with the Histone Promoter probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

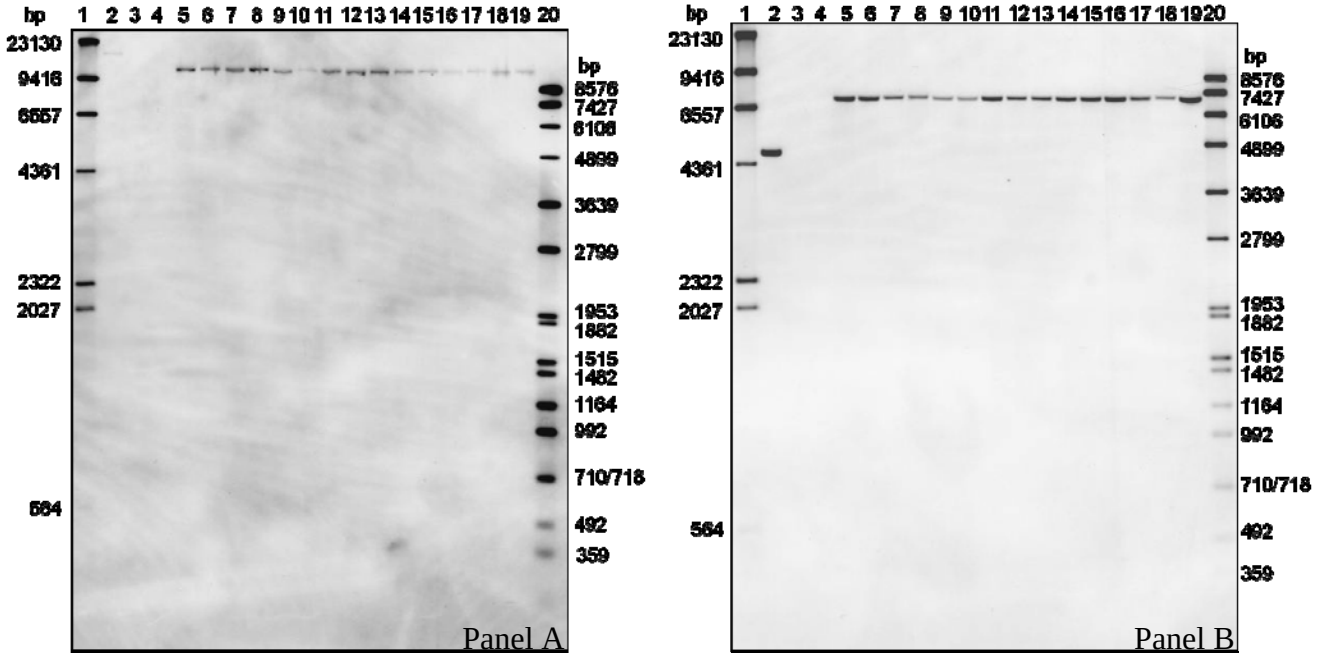
Figure 5. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A) and *Msc*I (Panel B) and Detected with the Histone Promoter Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A) and *Msc*I (Panel B) and hybridized with the AtUbi10 Promoter probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

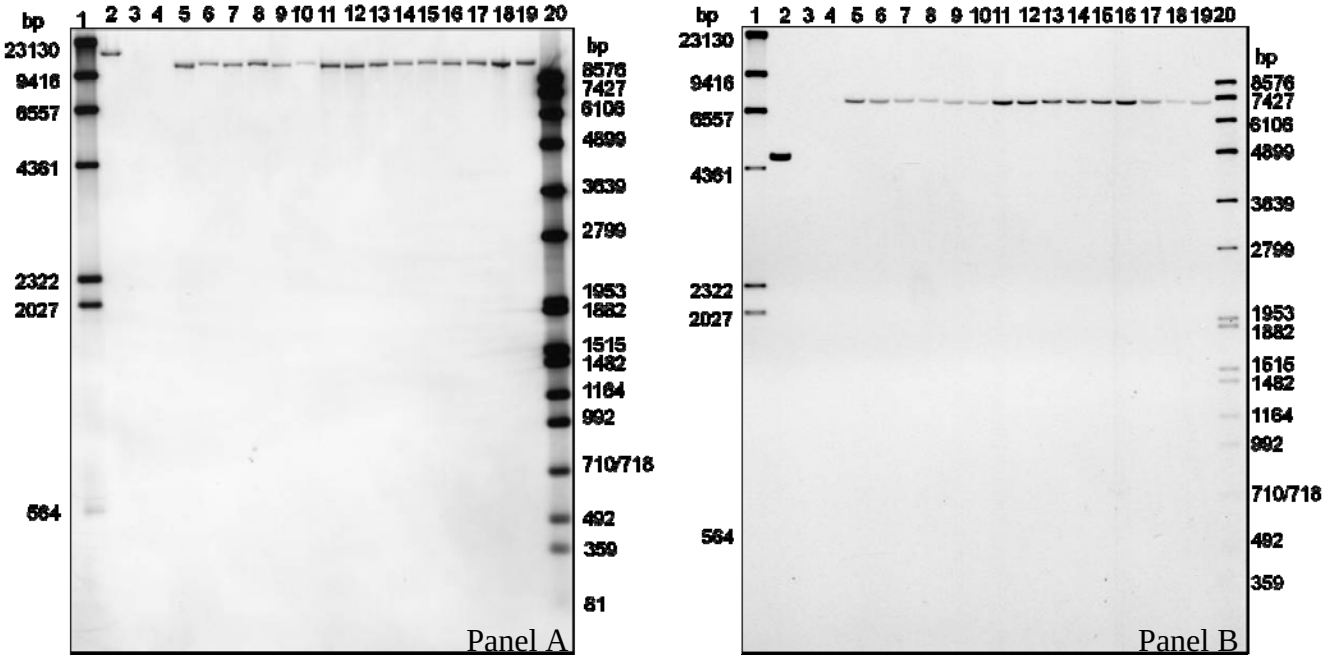
Figure 6. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A) and *Msc*I (Panel B) and Detected with the AtUbi10 Promoter Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A) and *Hin*dIII (Panel B) and hybridized with the AtuORF23 UTR probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

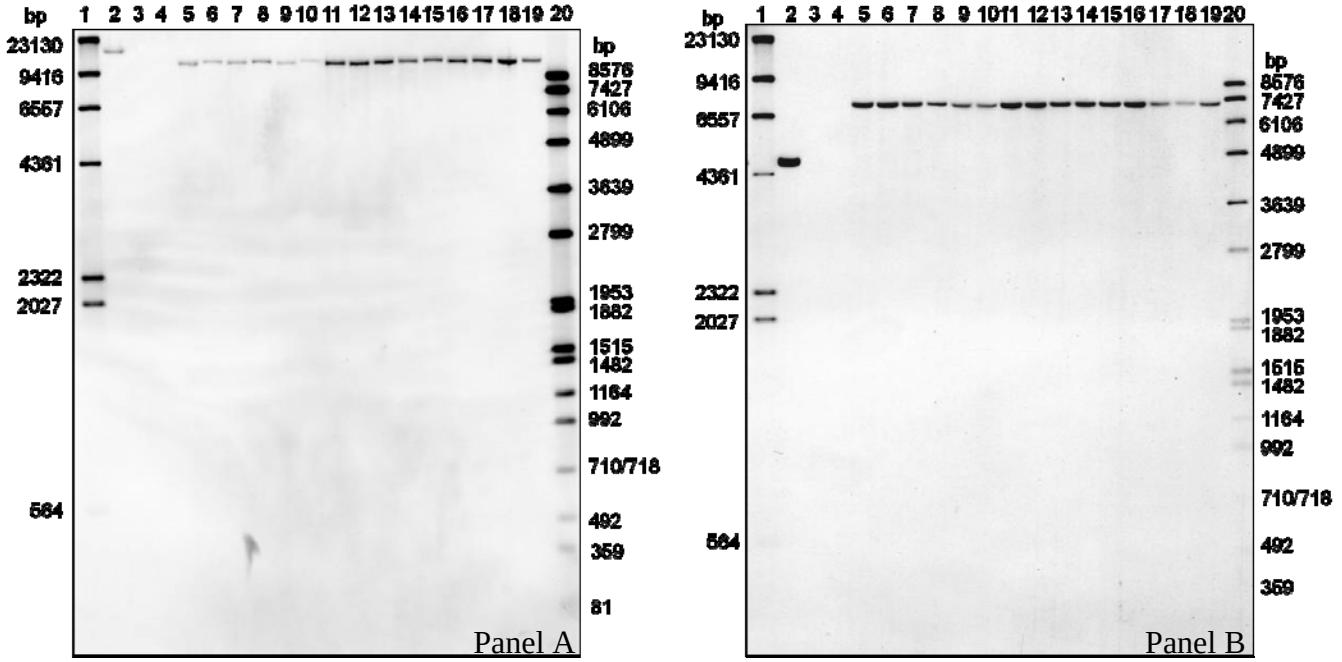
Figure 7. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A) and *Hin*dIII (Panel B) and Detected with the AtuORF23 UTR Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 μg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A) and *Hin*dIII (Panel B) and hybridized with the CsVMV probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

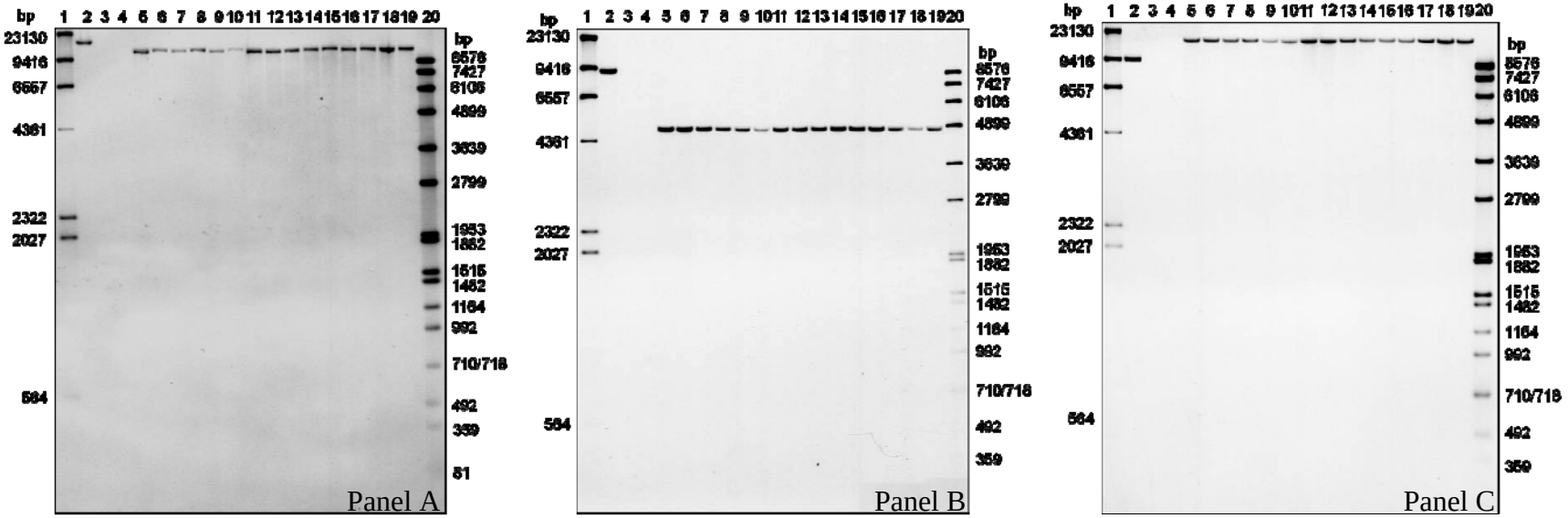
Figure 8. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A) and *Hin*dIII (Panel B) and Detected with the CsVMV Probe




| | Panel A | | | Panel B | | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A) and *Hin*dIII (Panel B) and hybridized with the AtuORF1 UTR probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

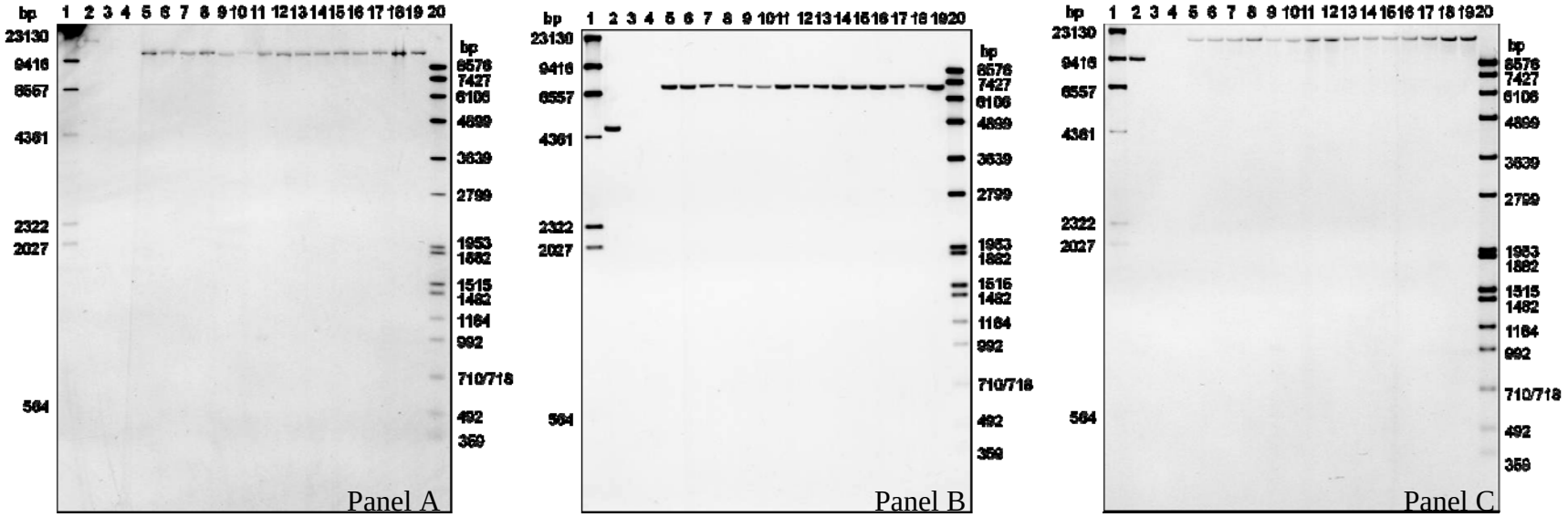
Figure 9. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A) and *Hin*dIII (Panel B) and Detected with the AtuORF1 UTR Probe




| | Panel A | | | | Panel B | | | | Panel C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | | 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | | 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | | 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | | 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | | 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | | 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | | 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | | 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | | 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A), *Hin*dIII (Panel B) and *Msc*I (Panel C) followed by hybridization with the *2mEPSPS* probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

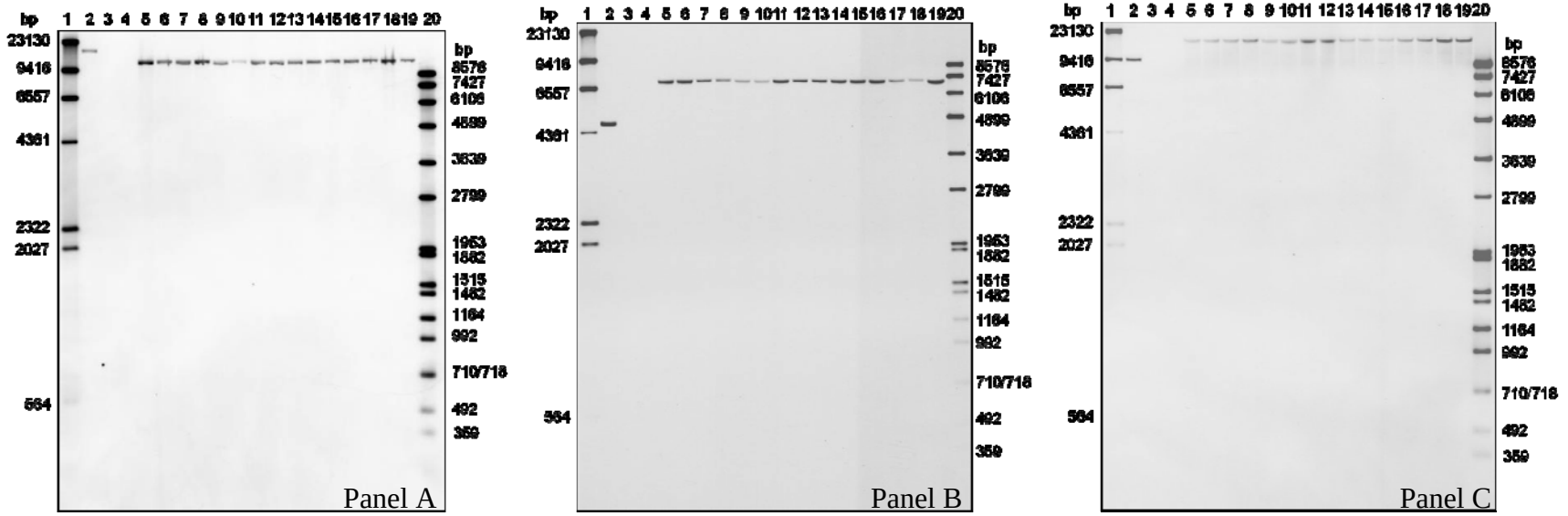
Figure 10. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A), *Hin*dIII (Panel B) and *Msc*I (Panel C) and Detected with the *2mEPSPS* Probe




| | Panel A | | | | Panel B | | | | Panel C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A), *Hin*dIII (Panel B) and *Msc*I (Panel C) followed by hybridization with the *aad*-12 probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

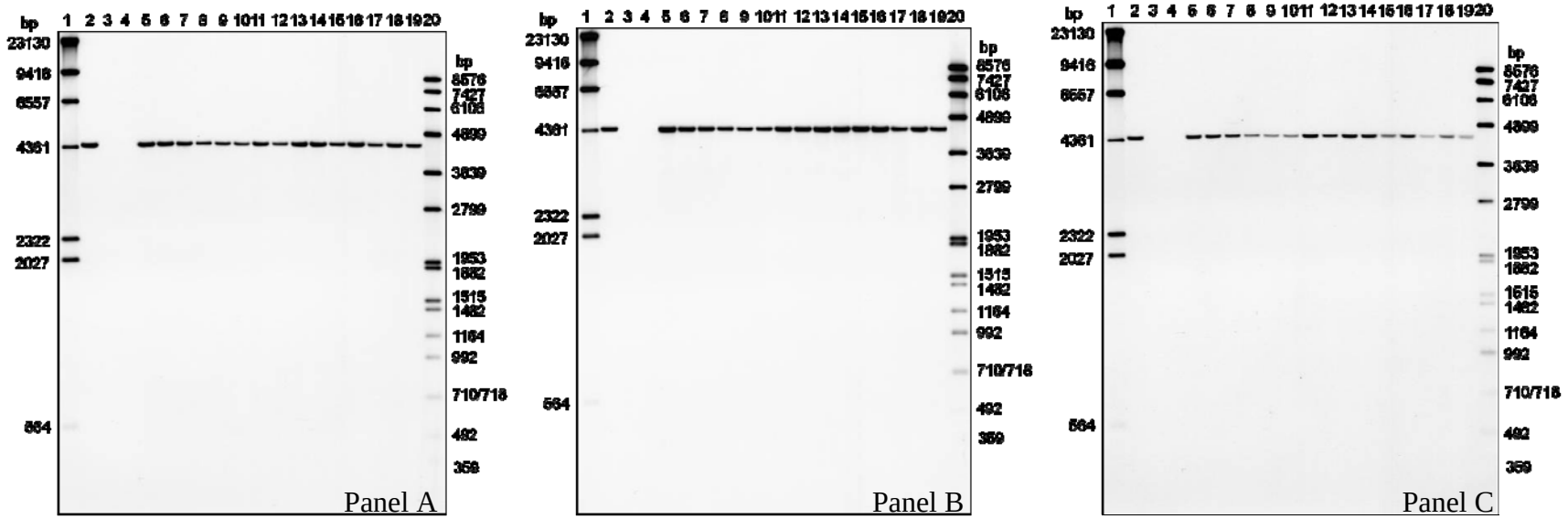
Figure 11. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A), *Hin*dIII (Panel B) and *Msc*I (Panel C) and Detected with the *aad*-12 Probe




| | Panel A | | | Panel B | | | | Panel C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Xho*I (Panel A), *Hin*dIII (Panel B) and *Msc*I (Panel C) followed by hybridization with the *pat* probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

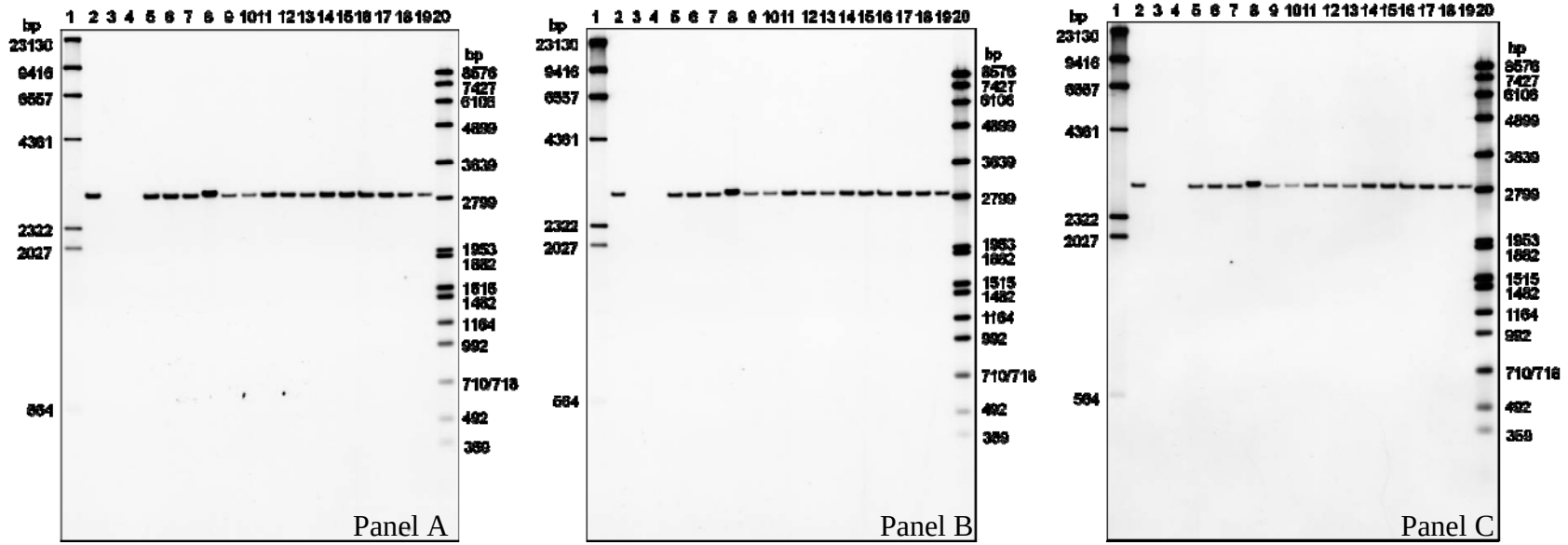
Figure 12. Southern Blot Analysis of DAS-44406-6 Digested with *Xho*I (Panel A), *Hin*dIII (Panel B) and *Msc*I (Panel C) and Detected with the *pat* Probe




| | Panel A | | | | Panel B | | | | Panel C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-3 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-3 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-3 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 | 3 | Maverick-3 | 13 | 4406-T3-3 |
| 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested *Msc*I/*Eco*RI followed by hybridization with the Histone Promoter (Panel A), *2mEPSPS* (Panel B) and Histone UTR (Panel C) probes. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

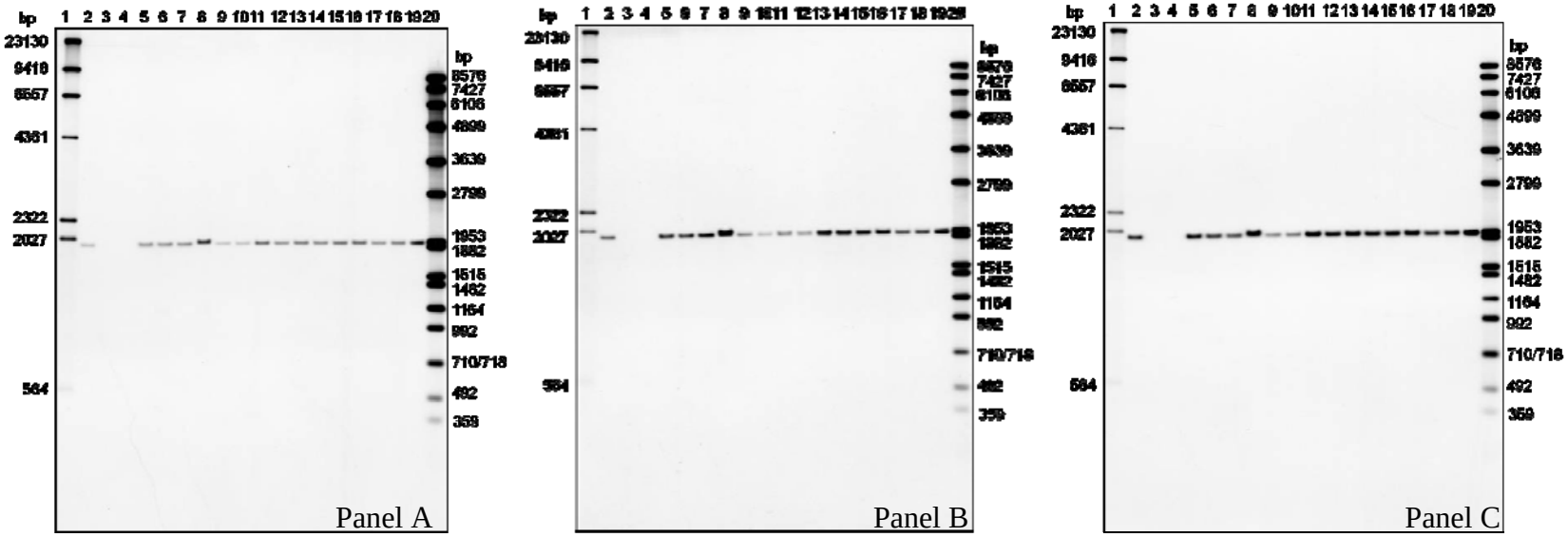
Figure 13. Southern Blot Analysis of DAS-44406-6 Digested with *Msc*I/*Eco*RI and Detected with the Histone Promoter (Panel A), *2mEPSPS* (Panel B) and Histone UTR (Panel C) Probes




| | Panel A | | | | Panel B | | | | Panel C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-1 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 |
| 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested *Pst*I/*Xho*I followed by hybridization with the AtUbi10 Promoter (Panel A), *aad*-12 (Panel B) and AtuORF23 UTR (Panel C) probes. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

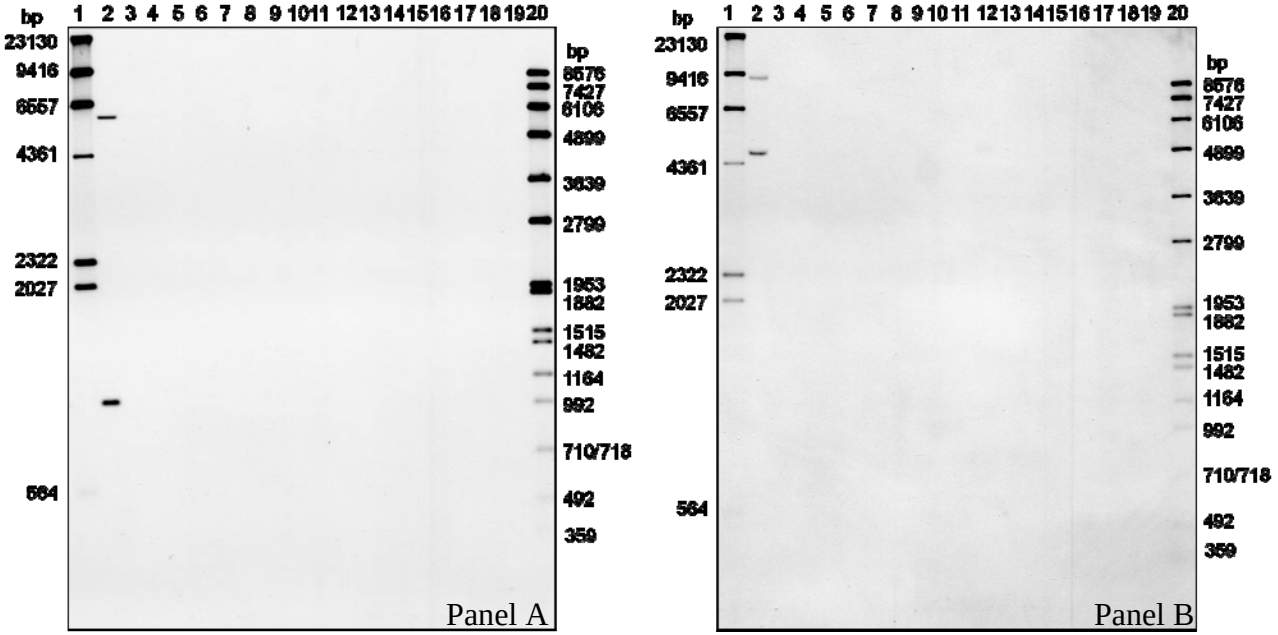
Figure 14. Southern Blot Analysis of DAS-44406-6 Digested with *Pst*I/*Xho*I and Detected with the AtUbi10 Promoter (Panel A), *aad*-12 (Panel B) and AtuORF23 UTR (Panel C) Probes




| | Panel A | | | Panel B | | | | Panel C | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-1 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 |
| 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested *Pst*I/*Xho*I followed by hybridization with the CsVMV (Panel A), *pat* (Panel B) and AtuORF1 UTR (Panel C) probes. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent image.

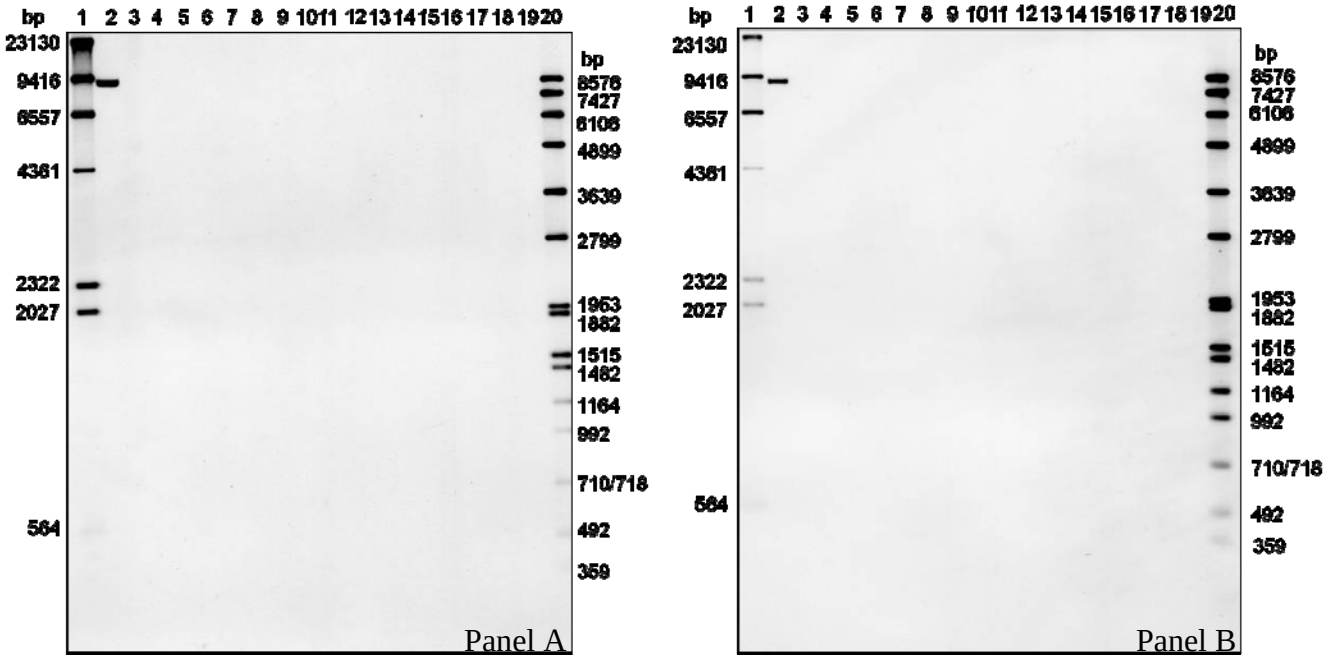
Figure 15. Southern Blot Analysis of DAS-44406-6 Digested with *Pst*I/*Xho*I and Detected with the CsVMV (Panel A), *pat* (Panel B) and AtuORF1 UTR (Panel C) Probes




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-1 | 13 | 4406-T3-3 | 3 | Maverick-5 | 13 | 4406-T3-3 |
| 4 | Maverick-2 | 14 | 4406-T4-1 | 4 | Maverick-4 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Msc*I/*Eco*RI (Panel A) and *Hin*dIII (Panel B) and hybridized with the Backbone 3 and Backbone 4 probes. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image. Note. In panel A, the Backbone 3 band is present at ~1000 bp, while the Backbone 4 is located at ~5900 bp. For panel B, the Backbone 3 band is present at ~4700 bp, while the Backbone 4 band is at ~9300 bp.

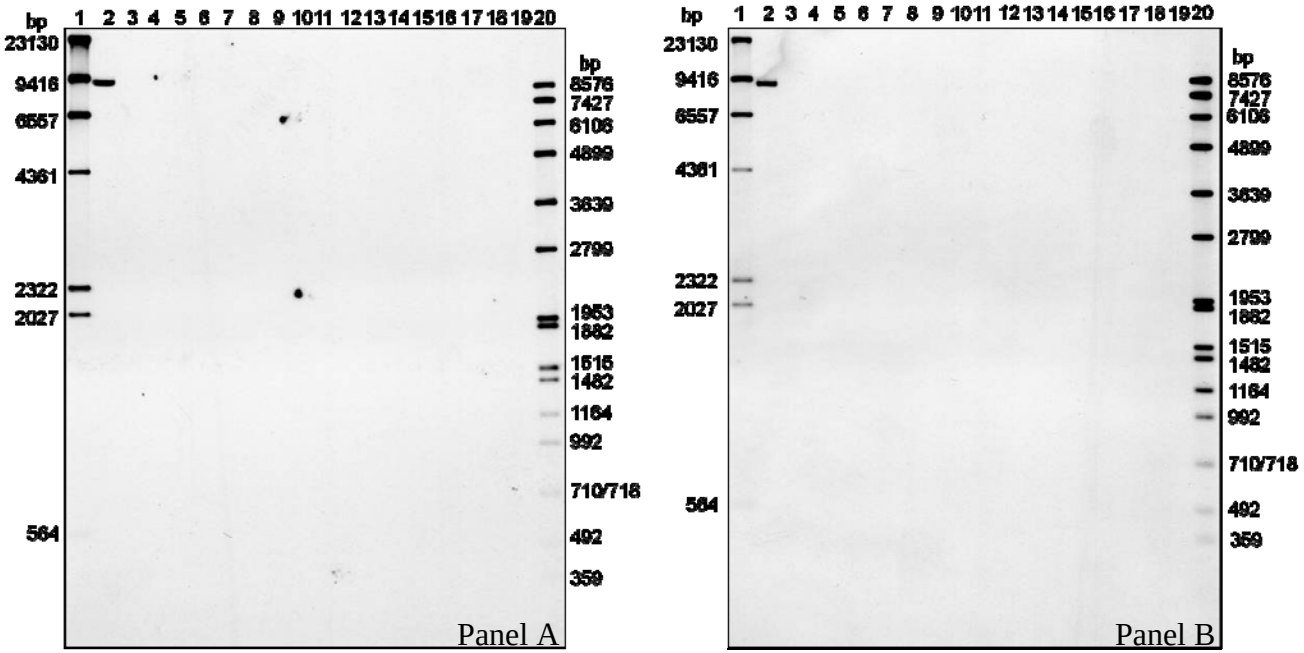
Figure 16. Southern Blot Analysis of DAS-44406-6 Digested with *Msc*I/*Eco*RI (Panel A) and *Hin*dIII (Panel B) and Detected with the Backbone 3 and Backbone 4 Probes




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and hybridized with the Ori-Rep probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

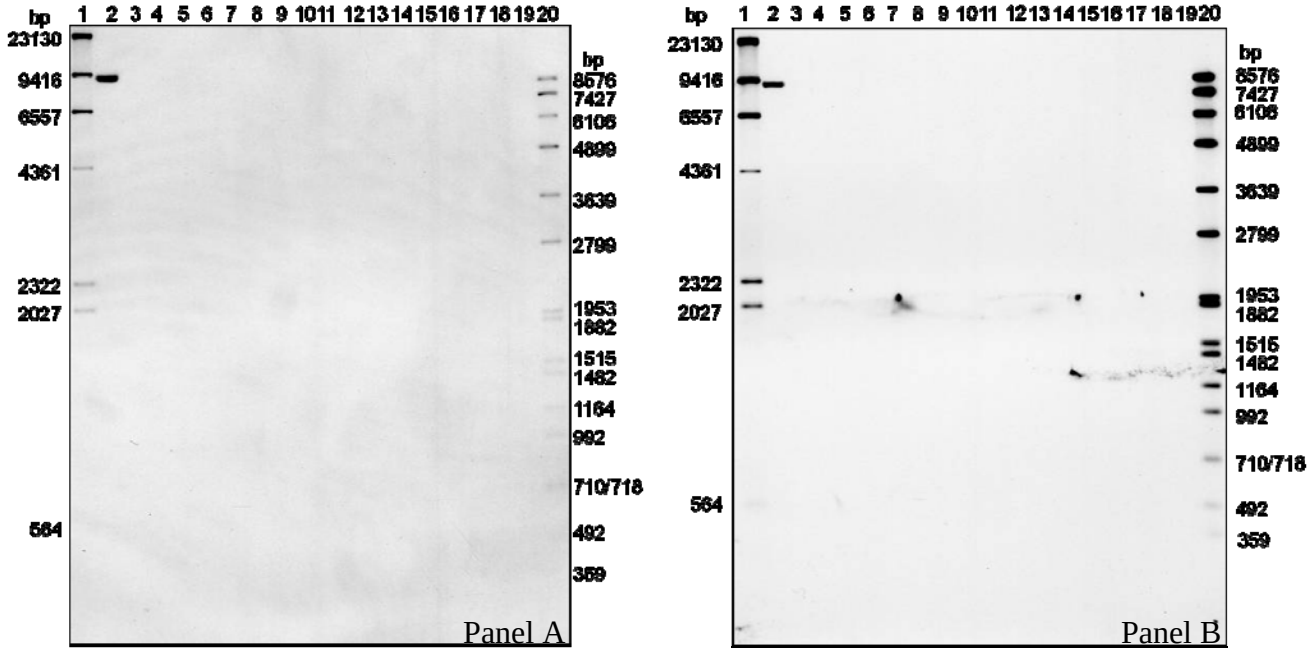
Figure 17. Southern Blot Analysis of DAS-44406-6 Digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and Detected with the Ori-Rep Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and hybridized with the Backbone 1 probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

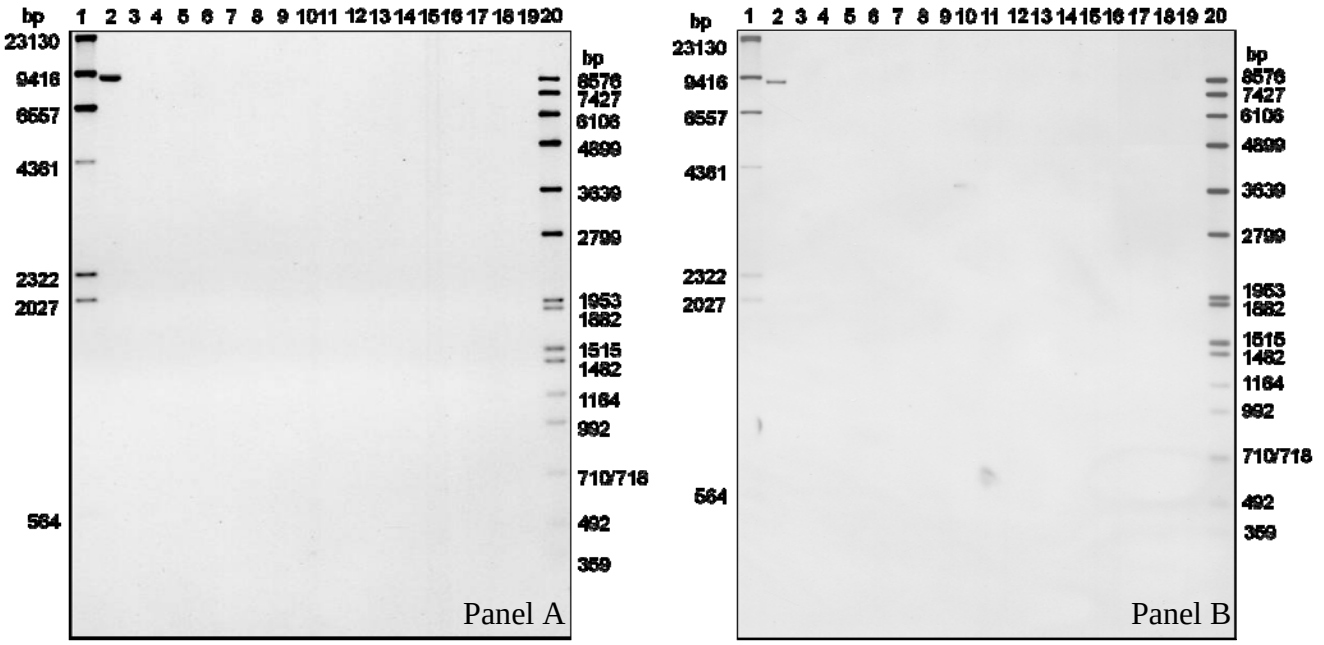
Figure 18. Southern Blot Analysis of DAS-44406-6 Digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and Detected with the Backbone 1 Probe




| | Panel A | | | | Panel B | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and hybridized with the Backbone 2 probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image.

Figure 19. Southern Blot Analysis of DAS-44406-6 Digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and Detected with the Backbone 2 Probe




| | Panel A | | | Panel B | | | |
|---|---|---|---|---|---|---|---|
| Lane | Sample | Lane | Sample | Lane | Sample | Lane | Sample |
| 1 | DIG MWM II | 11 | 4406-T3-1 | 1 | DIG MWM II | 11 | 4406-T3-1 |
| 2 | Maverick-5 + pDAB8264 | 12 | 4406-T3-2 | 2 | Maverick-1 + pDAB8264 | 12 | 4406-T3-2 |
| 3 | Maverick-5 | 13 | 4406-T3-3 | 3 | Maverick-1 | 13 | 4406-T3-3 |
| 4 | Maverick-4 | 14 | 4406-T4-1 | 4 | Maverick-2 | 14 | 4406-T4-1 |
| 5 | 4406-T2-1 | 15 | 4406-T4-4 | 5 | 4406-T2-1 | 15 | 4406-T4-4 |
| 6 | 4406-T2-2 | 16 | 4406-T4-5 | 6 | 4406-T2-2 | 16 | 4406-T4-5 |
| 7 | 4406-T2-3 | 17 | 4406-T6-1 | 7 | 4406-T2-3 | 17 | 4406-T6-1 |
| 8 | 4406-F2-1 | 18 | 4406-T6-2 | 8 | 4406-F2-1 | 18 | 4406-T6-2 |
| 9 | 4406-F2-3 | 19 | 4406-T6-3 | 9 | 4406-F2-3 | 19 | 4406-T6-3 |
| 10 | 4406-F2-4 | 20 | DIG MWM VII | 10 | 4406-F2-4 | 20 | DIG MWM VII |

Approximately 10 µg of DAS-44406-6 genomic DNA was digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and hybridized with the SpecR probe. Plasmid pDAB8264 served as the positive control at approximately one copy per soybean genome. Fragment sizes of the DIG-labeled DNA Molecular Weight Marker II and VII are indicated adjacent to image. *Note: The imperfection at ~4000 bp between lanes 9 and 10 in panel B is non-specific background signal since it falls between the lanes.*

Figure 20. Southern Blot Analysis of DAS-44406-6 Digested with *Hin*dIII (Panel A) *Pst*I/*Xho*I and (Panel B) and Detected with the SpecR Probe