## SUMMARY

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

## STUDY TITLE

Molecular Characterization of AAD-1 Corn Event DAS-40278-9

## DATA REQUIREMENTS

Not Applicable

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## STUDY COMPLETED ON

18-May-2009

## PERFORMING LABORATORY

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## LABORATORY STUDY ID

081052

# Molecular Characterization of AAD-1 Corn Event DAS-40278-9

## SUMMARY

Corn has been modified by the insertion of the *aad*-1 gene from *Sphingomonas herbicidivorans* which encodes the aryloxyalkanoate dioxygenase-1 (AAD-1) protein. The trait confers tolerance to 2,4-dicholrophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (e.g. haloxyfop, cyhalofop, quizalofop) herbicides and is also used as a selectable marker during plant transformation. Whiskers transformation of corn with a DNA fragment released by *Fsp* I from plasmid pDAS1740 (also known as pDAB3812) was carried forward, through breeding, to produce AAD-1 corn event DAS-40278-9 (pDAS1740-278) which was the focus of this study. Additionally, two similar copies of RB7 (Mar v3 and Mar v4), matrix attachment regions originally came from tobacco (*Nicotiana tabacum*), located at the 5' and 3' ends of the *aad*-1 PTU (plant transcription unit) region, were also included in pDAS1740/*Fsp* I fragment for transformation to facilitate gene expression. The initial transgenic event DAS-40278-9, carrying the insert from pDAS1740, has been bred into elite germplasm to stabilize agronomic performance.

Leaf samples from five distinct generations of the event DAS-40278-9 were used to conduct the Southern blot analysis for molecular characterization. The integration pattern was investigated using selected restriction enzyme digest and probe combinations to characterize the inserted gene, *aad-1*, as well as the non-coding regions including promoter, terminator of gene expression, and the matrix attachment regions.

Based on the Southern blot characterization of the DNA inserted into event DAS-40278-9, the results suggest that a single intact copy of the *aad-1* PTU has been integrated into event DAS-40278-9. The hybridization pattern is identical across all five generations, indicating that the insert is stable in the corn genome. Hybridization with probes covering the backbone region

beyond the pDAS1740/*Fsp* I transformation fragment from plasmid pDAS1740 confirms that no vector backbone sequences have been incorporated into the event DAS-40278-9.

STUDY TITLE

Molecular Characterization of AAD-1 Corn Event DAS-40278-9

DATA REQUIREMENTS

Not Applicable

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# STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

Compound: **Aryloxyalkanoate Dioxygenase-1**

Title: Molecular Characterization of AAD-1 Corn Event DAS-40278-9

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

Company: Dow AgroSciences LLC

Company Agent: Penny L. Hunst

Title: Regulatory Manager

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Date: 17-Mar-2009

*In the United States, the above statement supersedes all other statements of confidentiality that may occur elsewhere in this report.

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

# STATEMENT OF COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Title: Molecular Characterization of AAD-1 Corn Event DAS-40278-9

Study Initiation Date: July-08-2008

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

Organisation for Economic Co-Operation and Development
ENV/MC/CHEM(98)17, Paris January 26, 1998

All aspects of this study were conducted in accordance with the requirements for Good Laboratory Practice Standards, 40 CFR 160, 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 (Digoxigenin (DIG)-labeled DNA Molecular Size Marker II and 1kb plus DNA ladder) was unknown.

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

**Study ID:** 081052

**Title:** Molecular Characterization of AAD-1 Corn Events DAS-40278-9

**Study Initiation Date:** 08-July-2008 **Study Completion Date:** 18-May-2009

**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 |
|---|---|---|
| 01-July-2008 | 02-July-2008 | Protocol Review |
| 13-Aug-2008 | 13-Aug-2008 | Experimental Initiation |
| 29-Oct-2008 | 29-Oct-2008 | DNA Extraction |
| 16, 17, 27-30-Apr-2009 | 01-May-2009 | Raw Data and Report Review; Test Substance Container and Sample Verification |

**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.

Gaya Wickremsinhe 18 May 2009

Gaya Wickremsinhe, RQAP-GLP
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Molecular Characterization of AAD-1 Corn Event DAS-40278-9

## ABSTRACT

Corn has been modified by the insertion of the *aad*-1 gene from *Sphingomonas herbicidivorans* which encodes the aryloxyalkanoate dioxygenase-1 (AAD-1) protein. The trait confers tolerance to 2,4-dicholrophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (e.g. haloxyfop, cyhalofop, quizalofop) herbicides and is also used as a selectable marker during plant transformation. Whiskers transformation of corn with a DNA fragment released by *Fsp* I from plasmid pDAS1740 (also known as pDAB3812) was carried forward, through breeding, to produce AAD-1 corn event DAS-40278-9 (pDAS1740-278) which was the focus of this study. Additionally, two similar copies of RB7 (Mar v3 and Mar v4), matrix attachment regions originally came from tobacco (*Nicotiana tabacum*), located at the 5' and 3' end of the *aad*-1 PTU (plant transcription unit) region, were also included in pDAS1740/*Fsp* I fragment for transformation to facilitate gene expression. The initial transgenic event DAS-40278-9, carrying the insert from pDAS1740, has been bred into elite germplasm to stabilize agronomic performance.

Leaf samples from five distinct generations of the event DAS-40278-9 were used to conduct the Southern blot analysis for molecular characterization. The integration pattern was investigated using selected restriction enzyme digest and probe combinations to characterize the inserted gene, *aad-1*, as well as the non-coding regions including promoter, terminator of gene expression, and the matrix attachment regions.

Based on the Southern blot characterization of the DNA inserted into event DAS-40278-9, the results suggest that a single intact copy of the *aad-1* PTU has been integrated into event DAS-40278-9. The hybridization pattern is identical across all five generations, indicating that the insert is stable in the corn genome. Hybridization with probes covering the backbone region

beyond the pDAS1740/*Fsp* I transformation fragment from plasmid pDAS1740 confirms that no vector backbone sequences have been incorporated into the event DAS-40278-9.

# ABBREVIATIONS

| | |
|---|---|
| AAD-1 | aryloxyalkanoate dioxygenase-1 |
| bp | base pair |
| °C | degrees Celcius |
| DNA | deoxyribonucleic acid |
| DIG | digoxigenin |
| EDTA | ethylenediaminetetraacetic acid |
| kb | kilobase |
| µg | microgram |
| µL | microliter |
| mL | milliliter |
| M | molar mass |
| OLP | overlapping probe |
| PCR | polymerase chain reaction |
| PTU | plant transcription unit |
| SDS | sodium dodecyl sulfate |
| SOP | standard operating procedure |
| SSC | a buffer solution containing a mixture of sodium chloride and sodium citrate, pH 7.0 |
| TBE | a buffer solution containing a mixture of Tris base, boric acid and EDTA, pH 8.3 |
| V | volts |

# INTRODUCTION

Corn has been modified by the insertion of the *aad*-1 gene from *Sphingomonas herbicidivorans* which encodes the aryloxyalkanoate dioxygenase-1 (AAD-1) protein. The trait confers tolerance to 2,4-dicholrophenoxyacetic acid (2,4-D) and aryloxyphenoxypropionate (e.g. haloxyfop, cyhalofop, quizalofop) herbicides and can also be used as a selectable marker during plant transformation. Whiskers transformation of corn with plasmid pDAS1740 (also known as pDAB3812) was carried forward, through breeding, to produce AAD-1 corn event DAS-40278-9 (pDAS1740-278) which was the focus of this study.

This report describes the molecular characterization of the inserted DNA in AAD-1 corn event DAS-40278-9. The event was produced via Whiskers transformation with the *Fsp* I fragment of plasmid pDAS1740. Southern blot analysis was used to establish the integration pattern of the inserted DNA fragment and determine insert/copy number of the *aad-1* gene in event DAS-40278-9. Data were generated to demonstrate the integration and integrity of the *aad*-1 transgene inserted into the corn genome. Characterization of the integration of noncoding regions (designed to regulate the coding regions), such as promoters and terminators, the matrix attachment regions RB7 Mar v3 and RB7 Mar v4, as well as stability of the transgene insert across generations, were evaluated. The stability of the inserted DNA was demonstrated across five distinct generations of plants. Furthermore, absence of transformation plasmid backbone sequence including the Ampicillin resistance gene ($Ap^r$) region was demonstrated by probes covering nearly the whole backbone region flanking the restriction sites (*Fsp* I) of plasmid pDAS1740. A detailed physical map of the insertion has been hypothesized based on these Southern blot analyses of event DAS-40278-9.

Southern blot data suggested that the pDAS1740/*Fsp* I fragment insert in corn event DAS-40278-9 occurred as a simple integration of a single, intact copy of the *aad-1* PTU from plasmid pDAS1740. Detailed Southern blot analysis was conducted using probes specific to gene, promoter, terminator, and other regulation elements contained in the plasmid region and descriptive restriction enzymes that have cleavage sites located within the plasmid and produce hybridizing fragments internal to the plasmid or fragments that span the junction of the plasmid with corn genomic DNA (border fragments). These analyses established that the plasmid fragment had been inserted into corn genomic DNA without rearrangements of the *aad-1* PTU. Identical hybridization fragments were observed in five distinct generations of transgenic corn event DAS-40278-9 indicating stability of inheritance of the *aad-1* PTU insertion across generations. Hybridization with a mixture of three backbone probes located outside of the restriction site of *Fsp* I on plasmid pDAS1740 did not detect any specific gene fragments, indicating the absence of the Ampicillin resistance gene and the absence of the other vector backbone regions immediately adjacent to the *Fsp* I restriction sites of the plasmid pDAS1740 in transgenic corn event DAS-40278-9. The hypothesized map of the insert in AAD-1 corn event DAS-40278-9 is presented in Figures 2-3.

## MATERIALS AND METHODS

### Test Substance/Test system

The test substances and test systems were genomic DNA prepared from leaf of the individual plants of the AAD-1 corn event DAS-40278-9 (pDAS1740-278). Genomic DNA was extracted from leaf tissue harvested from individual plants carrying AAD-1 corn event DAS-40278-9. Transgenic corn seeds from five distinct generations of event DAS-40278-9, along with the source identification, were provided by the Department of TG&T in Dow AgroSciences (Table 1). Test substance was labeled with unique IDs associated with event number, plant generation, and plant number.

Control Substances

The control substance was genomic DNA prepared from leaf of the individual conventional corn XHH13. The conventional control plant has the genetic background representative of the test substance line, but does not contain the *aad*-1 gene. The conventional corn seeds were provided by the Department of TG&T in Dow AgroSciences (Table 1). Control substance was labeled with unique IDs associated with sample name and plant number.

Reference Materials

A 1 kb plus DNA ladder (Invitrogen, Cat #: 10787-018) and DIG DNA Marker II (Roche Diagnostics, Cat #: 11218590910), each contains a mixture of DNA fragments with different sizes, served as size references for agarose gel electrophoresis and Southern blot analysis.

The *Fsp* I fragment of plasmid pDAS1740 (pDAB3812) was used as the transformation DNA to generate AAD-1 corn event DAS-40278-9 (pDAS1740-278). pDAS1740 (pDAB3812) therefore serves as a positive control for the *aad*-1 transgene sequence in AAD-1 corn event DAS-40278-9 (pDAS1740-278). The reference plasmid was added to DNA samples from conventional corn plants for Southern blot analysis.

Seed Planting

Test and control corn seeds were planted in a Dow AgroSciences Indianapolis greenhouse with the pots uniquely identified by labeled stakes following the DAS procedure SOP-ECL-32a. The plants were grown under typical greenhouse conditions for corn. Ten to twenty pots, one seed per pot, were planted for each transgenic generation. Ten pots, one seed per pot, were planted for the conventional control XHH13. Emerged plants were labeled accordingly and were grown for at least 2 weeks prior to AAD-1 protein expression verification using lateral flow strips screening test.

Lateral Flow Strips Screening Test

Leaf punches were taken from each plant to test AAD-1 protein expression using a rapid test strip kit specifically for AAD-1 corn leaf (American Bionostica, Cat #: 701K100) according to the manufacturer's recommended procedure. Each leaf punch sample was given a score of + or – for the presence or absence of AAD-1, respectively. Only positive plants from the five generations of event DAS-40278-9 were subjected to further characterization.

Corn Leaf Sample Collection

Corn leaf samples were collected from five distinct generations of the individual plants of the AAD-1 corn event DAS-40278-9 and the conventional control XHH13. Leaf samples were quickly frozen in liquid nitrogen and stored at approximately -80°C until usage. Detail source information was listed in Table 1.

Genomic DNA Extraction

Individual genomic DNA was extracted from frozen corn leaf tissue following the standard CTAB method. When necessary, some of the genomic DNA were further purified with Qiagen Genomic-Tip (Qiagen, Cat #: 10243, 10262) following procedures recommended by manufacturer. Following extraction, the DNA was quantified spectrofluorometrically using Pico Green reagent (Invitrogen, Cat #: P7589). The DNA was then visualized on an agarose gel to confirm values from the Pico Green analysis and to determine the DNA quality.

DNA Probes

DNA probes specific to the *aad-1* gene and the other elements in the plasmid pDAS1740 (pDAB3812) were produced by polymerase chain reaction (PCR) amplification using pDAS1740 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, nine micrograms (9µg) of genomic DNA from the corn event DAS-40278-9 DNA sample and the conventional control were digested by adding approximately five to eleven 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 *Eco*R I, *Nco* I, *Sac* I, *Fse* I, and *Hin*d III were used for the digests (New England Biolabs, Cat #: R0101S, R0193L, R0156L, R0588L, and R0104L, respectively). The positive hybridization control sample was prepared by combining plasmid DNA pDAS1740 (pDAB3812) with genomic DNA from the conventional control at a ratio of approximately equivalent to 1 copy of transgene per corn genome, and digested using the same procedures and restriction enzyme as the test samples. DNA from the conventional corn control (XHH13) 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 (Edge BioSystems, Cat #:72641) and resuspended in 1× Blue Juice (Invitrogen, Cat#: 10816-015) 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 (Fisher Scientific, Cat #: BP1333) at 55-65 volts for approximately 18-22 hours to achieve fragment separation. The gels were stained with ethidium bromide (Invitrogen, Cat #: 15585-011) 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 Memelink, et al. (2). Briefly, following electrophoretic separation and visualization of the DNA fragments, the gels were depurinated with 0.25N HCl (Fisher Scientific, Cat #: 5A48) for approximately 15 minutes, and then exposed to a denaturing solution (AccuGENE, Cat #: 51228; Sigma, Cat #: N1531) for approximately 30 minutes followed by neutralizing solution (AccuGENE, Cat #: 51230) for at

least 30 minutes. Southern transfer was performed overnight onto nylon membranes (Roche Diagnostics, Cat #: 1417240) using a wicking system with 10×SSC (Sigma, Cat #: S6639). After transfer the membranes were washed in a 2× SSC solution and the DNA was bound 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-11]-dUTP, from fragments generated by primers specific to gene elements and other regions from plasmid pDAS1740. Generation of DNA probes by PCR synthesis was carried out using a PCR DIG Probe Synthesis Kit (Roche Diagnostics, Cat #: 11636090910) following the manufacturer's recommended procedures.

Labeled probes were analyzed by agarose gel electrophoresis to determine their quality and quantity. 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 using the procedures essentially as described for DIG Easy Hyb Solution (Roche Diagnostics, Cat #: 1603558). Briefly, nylon membrane blots with DNA fixed on were briefly washed in 2×SSC and prehybridized with 20-25 mL of prewarmed DIG Easy Hyb solution in hybridization bottles at approximately 50°C for a minimal of 30 minutes in a hybridization oven. The prehybridization solution were then decanted and replaced with 20 mL of prewarmed DIG Easy Hyb solution containing a desired amount of specific probes predenatured by boiling in water for 5 minutes. The hybridization step was then conducted at approximately 40-60°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 -20°C. These probes could be reused for 2-3 times according to the manufacturer's recommended procedure. The membrane blots were rinsed briefly and washed twice in clean plastic containers with low stringency wash buffer (2×SSC, 0.1%SDS) for approximately 5 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. The membrane blots were then transferred to other clean plastic containers and briefly washed with 1×washing buffer from the DIG Wash and Block Buffer Set (Roche Diagnostics, Cat #: 1585762) for approximately 2 minutes, proceeded to blocking in 1× blocking buffer for a minimum of 30 minutes, followed by incubation with anti-DIG-AP (alkaline phosphatase) antibody (Roche Diagnostics, Cat #: 11093274910, 1:5,000 dilution) in 1× blocking buffer for a minimum of 30 minutes. After 2-3 washes with 1× washing buffer, specific DNA probes remain bound to the membrane blots and DIG-labeled DNA standards were visualized using CDP-Star Chemiluminescent Nucleic Acid Detection System (Roche Diagnostics, Cat #: 11759051001) following the manufacturer's recommendation. Blots were exposed to chemiluminescent film (Roche Diagnostics, Cat #: 1666657) for one or more time points to detect hybridizing fragments and to visualize molecular size standards. Films were then developed with an All-Pro 100 Plus film developer (Konica SRX-101) and images were scanned for report. The number and sizes of detected bands were documented for each probe. DIG-labeled DNA Molecular Weight Marker II (MWM DIG II), visible after DIG detection as described, was used to determine hybridizing fragment size on the Southern blots.

Probe Stripping

DNA probes were stripped off 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 (Reference 3, Roche Diagnostics).

Briefly, after signal detection and film exposure, membrane blots were thoroughly rinsed with Milli-Q water and followed by washing twice in stripping buffer (0.2N NaOH, 0.1% SDS) for approximately 15 minutes at room temperature or at 37°C. The membrane blots were then briefly washed in 2×SSC and were ready for prehybridization and hybridization with another DNA probe. The membrane blots were exposed to a new chemiluminescent film to ensure all the DNA probes were stripped of before proceeding to the next hybridization. The re-exposed films were kept along with the previous hybridization data package in the study file for record.

## RESULTS AND DISCUSSION

Genomic DNA samples extracted from twenty individual corn plants derived from five generations and four plants per generation of event DAS-40278-9 (Table 1) were selected for molecular characterization of the AAD-1 corn event DAS-40278-9. AAD-1 protein expression was tested using an AAD-1 specific rapid test strip kit. Only plants that tested positive for AAD-1 protein expression were selected for subsequent molecular characterization. Southern hybridization confirmed that the *aad*-1 gene is present in corn plants that tested positive for AAD-1 protein expression (Table 1), and the *aad*-1 gene was inserted as a single intact copy in these plants when hybridized with an *aad*-1 gene probe.

Expected and observed fragment sizes with a particular digest and probe, based on the known restriction enzyme sites of the pDAS1740/*Fsp* I fragment, are given in Table 3. Two types of fragments were identified from these digests and hybridizations: internal fragments, where known enzyme sites flank the probe region and are completely contained within the pDAS1740/*Fsp* I fragment and border fragments where a known enzyme site is located at one end of the probe region and a second site is expected in the corn genome. Border fragment sizes vary by event because, in most cases, DNA fragment integration sites are unique for each event. The border fragments provide a means to locate a restriction enzyme site relative to the integrated DNA and to evaluate the number of DNA insertions. Based on the Southern blot analyses completed in this study, it was concluded that a single copy of an intact *aad*-1 PTU

from plasmid pDAS1740/*Fsp* I inserted into the corn genome of event DAS-40278-9 as detailed in the insert map (Figures 2-3).

Number of Insertion Sites

Restriction enzymes with unique restriction site in plasmid pDAS1740, *Eco*R I, *Nco* I, *Sac* I, *Fse* I/*Hin*d III, were selected to characterize *aad*-1 gene insert in event DAS-40278-9. Border fragment of > 3382 bp, >2764 bp, >4389 bp was predicted to hybridize with the *aad*-1 gene probe following *Eco*R I, *Nco* I, and *Sac* I digest respectively (Table 3). Single *aad*-1 hybridization band of ~12000 bp, ~4000 bp, and ~16000 bp were observed when *Eco*R I (Figures 4 &5), *Nco* I (Figures 6 &7), and *Sac* I (Figures 8 &9) were used respectively, indicating a single site of *aad*-1 gene insertion in the corn genome of event DAS-40278-9. Double digestion with *Fse* I and *Hin*d III was selected to release a fragment of 3361 bp which contains the *aad*-1 plant transcription unit (PTU, promoter/gene/terminator) (Table 3). The predicted 3361 bp fragment was observed with the *aad*-1 gene probe following *Fse* I/*Hin*d III digestion (Figures 10 &11). Results obtained with all four enzymes/enzyme combination digestion of the DAS-40278-9 sample followed by *aad*-1 gene probe hybridization indicated that a single copy of an intact *aad*-1 PTU from plasmid pDAS1740 was inserted into the corn genome of event DAS-40278-9.

Structure of the Insert and Its Genetic Elements

Restriction enzymes *Nco* I, *Sac* I and *Fse* I/*Hin*d III were selected to characterize the promoter (ZmUbi1) region for *aad*-1 in event DAS-40278-9. *Nco* I and *Sac* I digests are expected to generate a border region fragment of >3472 bp and >4389 bp, respectively, when hybridized to DNA probes specifically to the ZmUbi1 promoter region (Table 3). Two hybridization bands of ~6300 bp and ~3600 bp were detected with ZmUbi1 promoter probe following *Nco* I digestion (Figures 12 & 13). The ~3600 bp band, however, was present across all sample lanes including the conventional controls, suggesting that the ~3600 bp band is a non-specific signal band resulting from the homologous binding of the corn-derived ubiquitin promoter (ZmUbi1) probe

to the corn endogenous *ubi* gene. On the contrary, the ~6300 bp signal band was detected in the tested DAS-40278-9 samples but not in the conventional controls, indicating that the ~6300 bp band is specific to the ZmUbi1 promoter probe from plasmid pDAS1740 and therefore it is the expected *Nco* I/ZmUbi1 band indicated in Table 3. Similarly, two hybridization bands of ~3800 bp and ~16000 bp were detected with ZmUbi1 promoter probe following *Sac* I digestion (Figures 14 & 15). The ~3800 bp band appeared in all sample lanes including conventional controls and thus is considered as non-specific hybridization of ZmUbi1 promoter probe to the corn endogenous *ubi* gene. The ~16000 bp hybridization band that is only present in DAS-40278-9 samples is considered the expected *Sac* I/ ZmUbi1 band. Double digestion with *Fse* I/ *Hin*d III is expected to release the *aad*-1 PTU fragment of 3361 bp that hybridizes to the ZmUbi1 promoter probe (Table 3). This 3361 bp band and a non-specific hybridization band of ~6400 bp were detected by ZmUbi1 promoter probe following *Fse* I/ *Hin*d III digestion (Figures 16 & 17). The ~6400 bp band is considered non-specific binding of the ZmUbi1 promoter probe to the corn endogenous *ubi* gene because this band is present in all sample lanes including the conventional controls. Additionally, another band very close to ~6400 bp was observed in the conventional control, BC3S1 (Figure 16), and some of the BC3S2 (Figure 17) samples. This additional band very close to ~6400 bp is also considered non-specific because it is present in the conventional control XHH13 sample lanes and is most likely associated with the genetic background of XHH13.

Same restriction enzymes/enzyme combination, *Nco* I, *Sac* I and *Fse* I/*Hin*d III were selected to characterize the terminator (ZmPer5) region for *aad*-1 in event DAS-40278-9. *Nco* I digest is expected to generate a border region fragment of >2764 bp when hybridized to DNA probes specifically to the ZmPer5 terminator region (Table 3). Two hybridization bands of ~4000 bp and ~3900 bp were detected with ZmPer5 terminator probe following *Nco* I digestion (Figures 18 & 19). The ~3900 bp band was present across all sample lanes including the conventional controls, suggesting that the ~3900 bp band is a non-specific signal band probably due to the homologous binding of the corn-derived peroxidase gene terminator (ZmPer5) probe to the corn endogenous *per* gene. On the contrary, the ~4000 bp signal band was detected in the tested DAS-40278-9 samples but not in the conventional controls, indicating that the ~4000 bp band is

specific to the ZmPer5 terminator probe from plasmid pDAS1740 and therefore it is the expected *Nco* I/ZmPer5 band indicated in Table 3. A >1847 bp border fragment is expected to hybridized to the ZmPer5 terminator probe following *Sac* I digestion. Two hybridization bands of ~1900 bp and ~9000 bp were detected with ZmPer5 terminator probe following *Sac* I digestion (Figures 20 & 21). The ~9000 bp band appeared in all sample lanes including conventional controls and thus considered as non-specific hybridization of ZmPer5 terminator probe to the corn endogenous *per* gene. The ~1900 bp hybridization band that was only present in DAS-40278-9 samples is considered the expected *Sac* I/ ZmPer5 band. Double digestion with *Fse* I/ *Hin*d III is expected to release the *aad*-1 PTU fragment of 3361 bp that hybridizes to the ZmPer5 terminator probe (Table 3). This 3361 bp band and an additional non-specific hybridization band of ~2100 bp were detected by ZmPer5 terminator probe following *Fse* I/ *Hin*d III digestion (Figures 22 & 23). The additional ~2100 bp band is the non-specific binding of the ZmPer5 terminator probe to the corn endogenous gene since this band is present in all sample lanes including the negative controls. Results obtained with these digestions of the DAS-40278-9 sample followed by ZmUbi1 promoter and ZmPer5 terminator probe hybridization further confirmed that a single copy of an intact *aad*-1 PTU from plasmid pDAS1740 was inserted into the corn genome of event DAS-40278-9.

Restriction enzymes, *Nco* I and *Sac* I, were selected to characterize the rest of the components from pDAS1740/*Fsp* I fragment in AAD-1 corn event DAS-40278-9 (Table 3). DNA sequences of components RB7 Mar v3 and RB7 Mar v4 have over 99.7% identity, therefore DNA probes specific for RB7 Mar v3 or RB7 Mar v4 were expected to hybridize to DNA fragments containing either version of the RB7 Mar. Two border fragments of >2764 bp and >3472 bp were expected to hybridize with RB7 Mar v4 and RB7 Mar v3 probes following *Nco* I digestion (Table 3). Two hybridization bands of ~4000 bp and ~6300 bp were observed with either RB7 Mar v4 (Figures 24 & 25) or RB7 Mar v3 (Figures 28 & 29) probe after *Nco* I digestion in DAS-40278-9 samples. Similarly, two border fragments of >1847 bp and >4389 bp were predicted with RB7 Mar v4 and RB7 Mar v3 probes following *Sac* I digestion (Table 3). Hybridization bands of ~1900 bp and ~16000 bp were detected in DAS-40278-9 samples with RB7 Mar v4 (Figures 26 & 27) or RB7 Mar v3 (Figures 30 & 31) probe after *Sac* I digestion.

Taken together, the Southern hybridization results obtained with these element probes indicated that the DNA inserted in corn event DAS-40278-9 contains an intact *aad*-1 PTU along with the matrix attachment regions RB7 Mar v3 and RB7 Mar v4 at the 5' and 3' ends of the insert, respectively.

Absence of Backbone Sequences

Equal molar ratio combination of three DNA fragments (Table 2) covering nearly the entire *Fsp* I backbone region (4867-7143 bp in plasmid pDAS1740) of plasmid pDAS1740 were used as the backbone probe to characterize AAD-1 corn event DAS-40278-9. Plasmid pDAS1740/*Fsp* I fragment was used to generate event DAS-40278-9, therefore, no specific hybridization signal was expected with the backbone probe combination (Table 3) following any restriction enzyme digestion. Figures 32-35 confirmed that no specific hybridization signal was detected with backbone probe following *Nco* I or *Sac* I digestion in all DAS-40278-9 samples. The positive control lanes contained the expected hybridizing bands demonstrating that the probes were capable of hybridizing to any homologous DNA fragments if present in the samples. The data suggested that the insertion in corn event DAS-40278-9 did not include any vector backbone sequence outside of the *Fsp* I region from plasmid pDAS1740.

## CONCLUSIONS

The Southern blot data developed in this study suggested that the insert in AAD-1 corn event DAS-40278-9 resulted from a single insertion of one intact copy of the *aad*-1 PTU from plasmid pDAS1740 at one locus in the corn genome. Identical fragment sizes were observed with all enzyme and probe combinations for four plants each from five distinct generations for corn event DAS-40278-9, indicating stability of inheritance across generations. In addition, the results did not indicate any rearrangements of the *aad*-1 PTU fragment, as all expected internal restriction enzyme sites appeared to be intact and produced hybridizing fragments of expected size (Table 3). Moreover, the absence of the Ampicillin resistance gene ($Ap^r$) and other backbone regions

outside the *Fsp* I sites from plasmid pDAS1740 was confirmed and suggested that only DNA contained within the pDAS1740/*Fsp* I fragment was integrated into corn event DAS-40278-9.

## 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

1. Perkin Elmer, Molecular Biology Assistant 2000 User's Guide, pages 17-23.
2. Memelink, J.; Swords, K.; Harry J.; Hoge, C.; (1994) Southern, Northern, and Western Blot Analysis. Plant Mol. Biol. Manual F1:1-23.
3. DIG Application Manual for Filter Hybridization, (2003). Roche Diagnostics.

Table 1. Description of Seed Sources for AAD-1 Corn Event DAS-40278-9 (pDAS1740-278) and Control Used in the Study.

| Generation | Source ID | Plant ID | AAD-1 Protein Expression | Purpose |
|---|---|---|---|---|
| T3 | ZQ06LQ491122.001 | 1, 4, 13, 14 | positive | Test Substance |
| T4 | ZQ07CQ514766.003 | 1, 3, 5, 6 | positive | Test Substance |
| BC3S1 | ZQ07GQ546693.026 | 6, 8, 11, 12 | positive | Test Substance |
| BC3S2 | ZQ07LQ570702.110 | 1, 2, 3, 5 | positive | Test Substance |
| BC3S3 | ZW08EW011463.009 | 2, 3, 5, 7 | positive | Test Substance |
| XHH13 | ZQ07LQ573115 | 1,2,4,5 | negative | Control Substance |

Table 2. Location and Length of Probes used in Southern Analysis.

| Probe Name | Genetic Element | Position on pDAS1740 (bp) | Length (bp) |
|---|---|---|---|
| OLP1-3 | ubiquitin promoter (ZmUbi1) | 28-2123 | 2096 |
| OLP2 | *aad-1* gene | 2103-3022 | 920 |
| OLP3A | peroxidase terminator (ZmPer5) | 3002-3397 | 396 |
| OLP3B | RB7 Mar v4 | 3375-4865 | 1491 |
| OLP4ABC | Backbone (OLP4A) | 4900-5848 | 949 |
| | Backbone $Ap^r$ gene (OLP4B) | 5828-6681 | 855 |
| | Backbone (OLP4C) | 6660-7144 | 485 |
| OLP5-2 | RB7 Mar v3 | 7124-8507 | 1384 |

Table 3. Predicted and Observed Hybridizing Fragments in Southern Blot Analysis.

| DNA Probe | Restriction Enzymes | | Figures | Expected Fragment Sizes (bp) [1] | Observed Fragment Size (bp)[2] |
|---|---|---|---|---|---|
| *aad-1* | *Eco*R I | pDAS1740 | 4,5 | 8512 | 8512 |
| | | XHH13 | 4,5 | none | none |
| | | DAS-40278-9 | 4,5 | >3382 (border) | ~12000 |
| | *Nco* I | pDAS1740 | 6,7 | 8512 | 8512 |
| | | XHH13 | 6,7 | none | none |
| | | DAS-40278-9 | 6,7 | >2764 (border) | ~4000 |
| | *Sac* I | pDAS1740 | 8,9 | 8512 | 8512 |
| | | XHH13 | 8,9 | none | none |
| | | DAS-40278-9 | 8,9 | >4389 (border) | ~16000 |
| | *Fse* I / *Hin*d III | pDAS1740 | 10,11 | 3361 | 3361 |
| | | XHH13 | 10,11 | none | none |
| | | DAS-40278-9 | 10,11 | 3361 | 3361 |
| | | | | | |
| ZmUbi1 prom. | *Nco* I | pDAS1740 | 12,13 | 8512 | 8512, ~3600* |
| | | XHH13 | 12,13 | none | ~3600* |
| | | DAS-40278-9 | 12,13 | >3472 (border) | ~6300, ~3600* |
| | *Sac* I | pDAS1740 | 14,15 | 8512 | 8512, ~3800* |
| | | XHH13 | 14,15 | none | ~3800* |
| | | DAS-40278-9 | 14,15 | >4389 (border) | ~3800*, ~16000 |
| | *Fse* I / *Hin*d III | pDAS1740 | 16,17 | 3361 | 3361, ~6400* |
| | | XHH13 | 16,17 | none | ~6400* |
| | | DAS-40278-9 | 16,17 | 3361 | 3361, ~6400*# |
| | | | | | |
| ZmPer5 term. | *Nco* I | pDAS1740 | 18,19 | 8512 | 8512, ~3900* |
| | | XHH13 | 18,19 | none | ~3900* |
| | | DAS-40278-9 | 18,19 | >2764 (border) | ~4000, ~3900* |
| | *Sac* I | pDAS1740 | 20,21 | 8512 | 8512, ~9000* |
| | | XHH13 | 20,21 | none | ~9000* |
| | | DAS-40278-9 | 20,21 | >1847 (border) | ~1900, ~9000* |
| | *Fse* I / *Hin*d III | pDAS1740 | 22,23 | 3361 | 3361, ~2100* |
| | | XHH13 | 22,23 | none | ~2100* |
| | | DAS-40278-9 | 22,23 | 3361 | 3361, ~2100* |

(Cont. ) Table 3. Predicted and Observed Hybridizing Fragments in Southern Blot Analysis

| DNA Probe | Restriction Enzymes | | Figures | Expected Fragment Sizes (bp) [1] | Observed Fragment Size (bp)[2] |
|---|---|---|---|---|---|
| RB7 mar4 | *Nco* I | pDAS1740 | 24,25 | 8512 | 8512 |
| | | XHH13 | 24,25 | none | none |
| | | DAS-40278-9 | 24,25 | >2764 (border)<br>>3472 (border) | ~4000<br>~6300 |
| | *Sac* I | pDAS1740 | 26,27 | 8512 | 8512 |
| | | XHH13 | 26,27 | none | none |
| | | DAS-40278-9 | 26,27 | >1847 (border)<br>>4389 (border) | ~1900<br>~16000 |
| | | | | | |
| RB7 mar3 | *Nco* I | pDAS1740 | 28,29 | 8512 | 8512 |
| | | XHH13 | 28,29 | none | none |
| | | DAS-40278-9 | 28,29 | >2764 (border)<br>>3472 (border) | ~4000<br>~6300 |
| | *Sac* I | pDAS1740 | 30,31 | 8512 | 8512 |
| | | XHH13 | 30,31 | none | none |
| | | DAS-40278-9 | 30,31 | >1847 (border)<br>>4389 (border) | ~1900<br>~16000 |
| | | | | | |
| backbone | *Nco* I | pDAS1740 | 32,33 | 8512 | 8512 |
| | | XHH13 | 32,33 | none | none |
| | | DAS-40278-9 | 32,33 | none | none |
| | *Sac* I | pDAS1740 | 34,35 | 8512 | 8512 |
| | | XHH13 | 34,35 | none | none |
| | | DAS-40278-9 | 34,35 | none | none |

Note: * An asterisk after the observed fragment size indicates endogenous sequence hybridization that was detected across all samples (including negative controls)
# Doublets in the conventional control, BC3S1, and some BC3S2 samples

1. Expected fragment sizes are based on the plasmid map of the pDAS1740 (pDAB3812) as shown in Figure 1.
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 II 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.

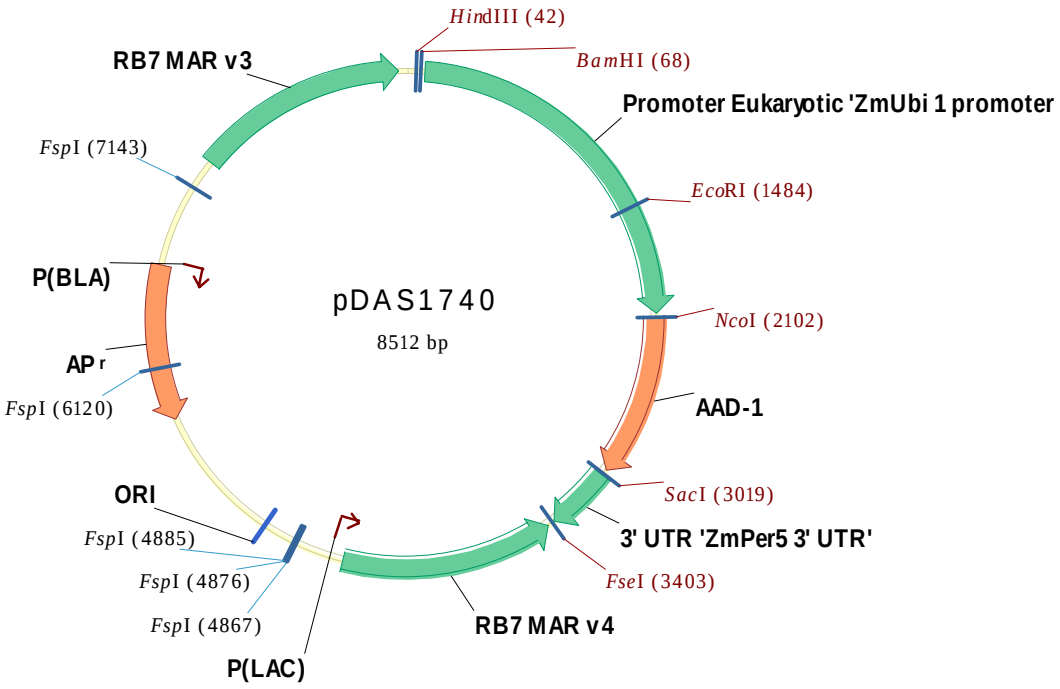



Figure 1. Plasmid Map of pDAS1740 (pDAB3812) with Restriction Enzyme Sites.

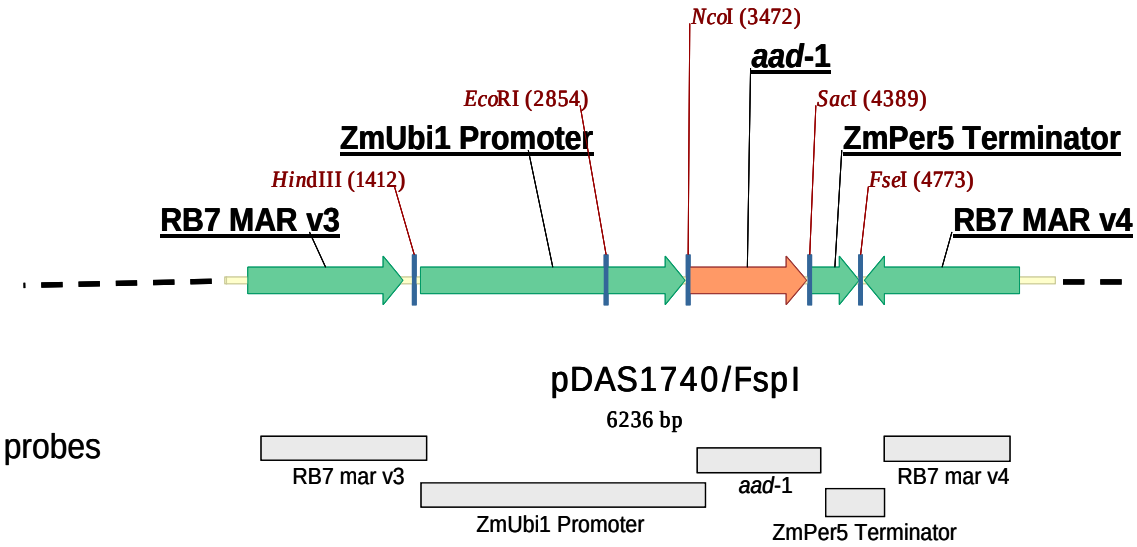



Figure 2. Restriction Map of the Single Insertion Site Present in the Genome of DAS-40278-9 (pDAS1740-278) Maize.

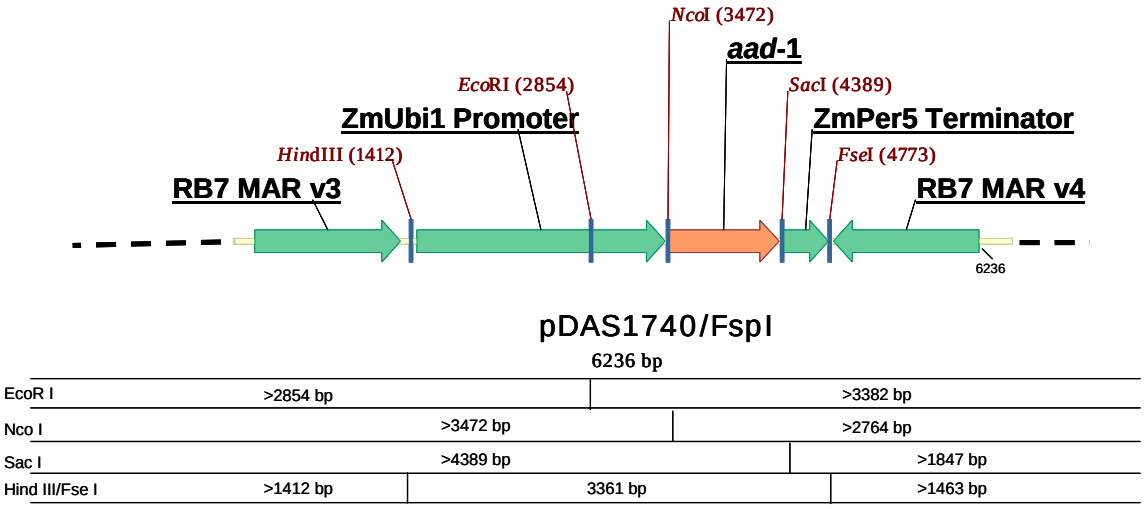
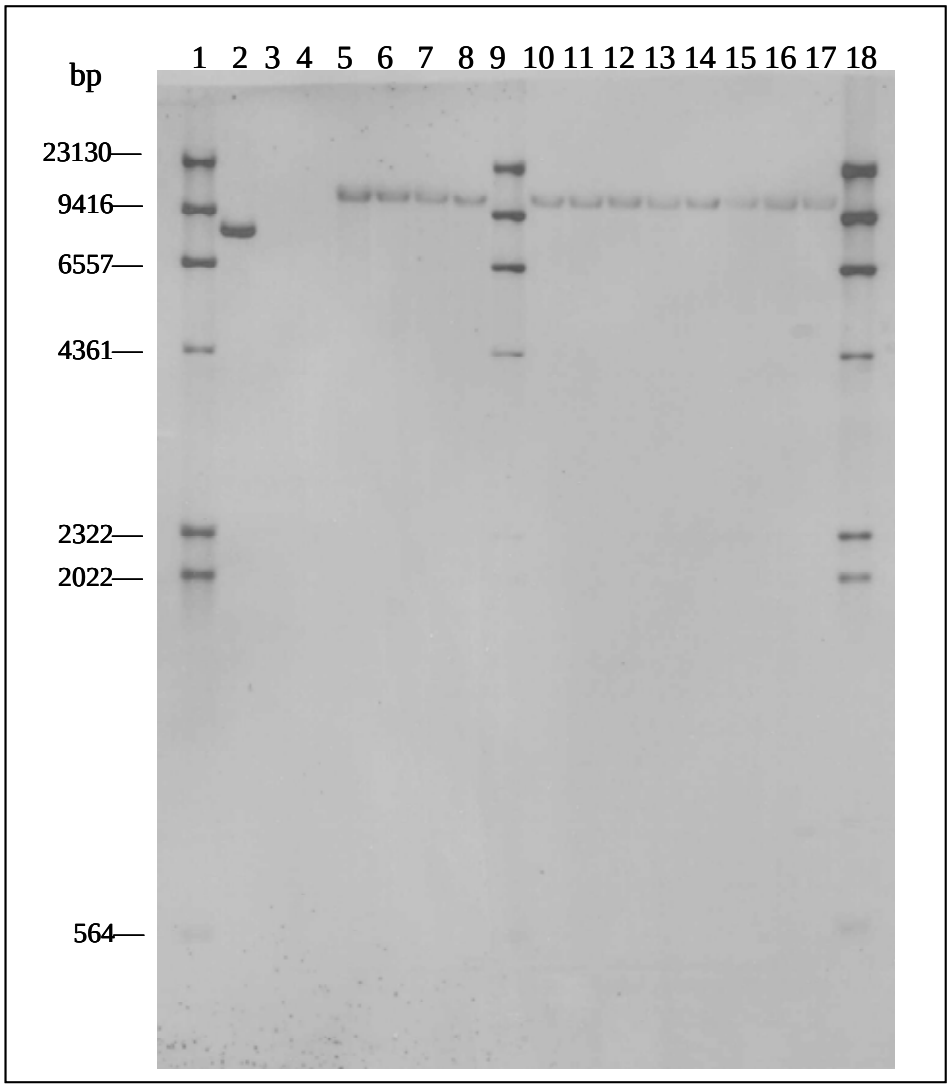


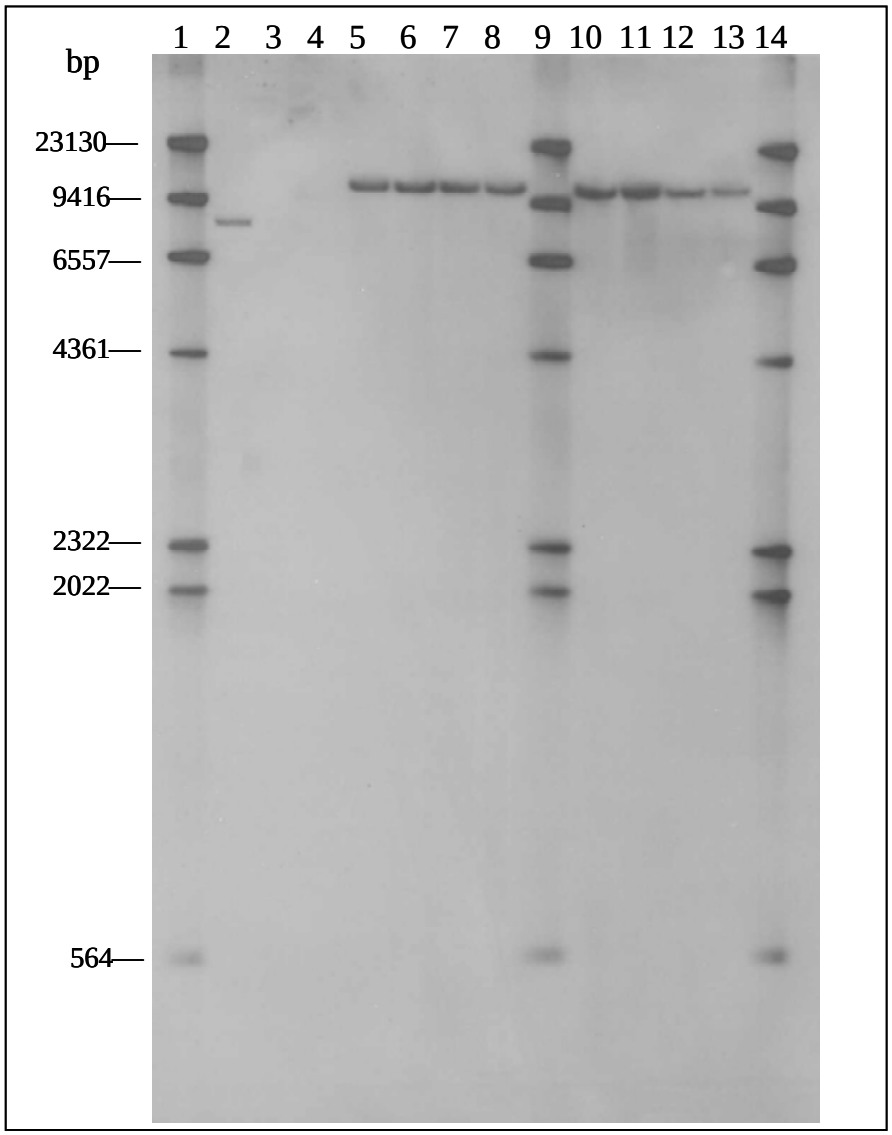

Figure 3. Restriction Map of the Single Insertion Site Present in the Genome of DAS-40278-9 Maize.




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Eco*R I and probed with the *aad*-1 gene probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

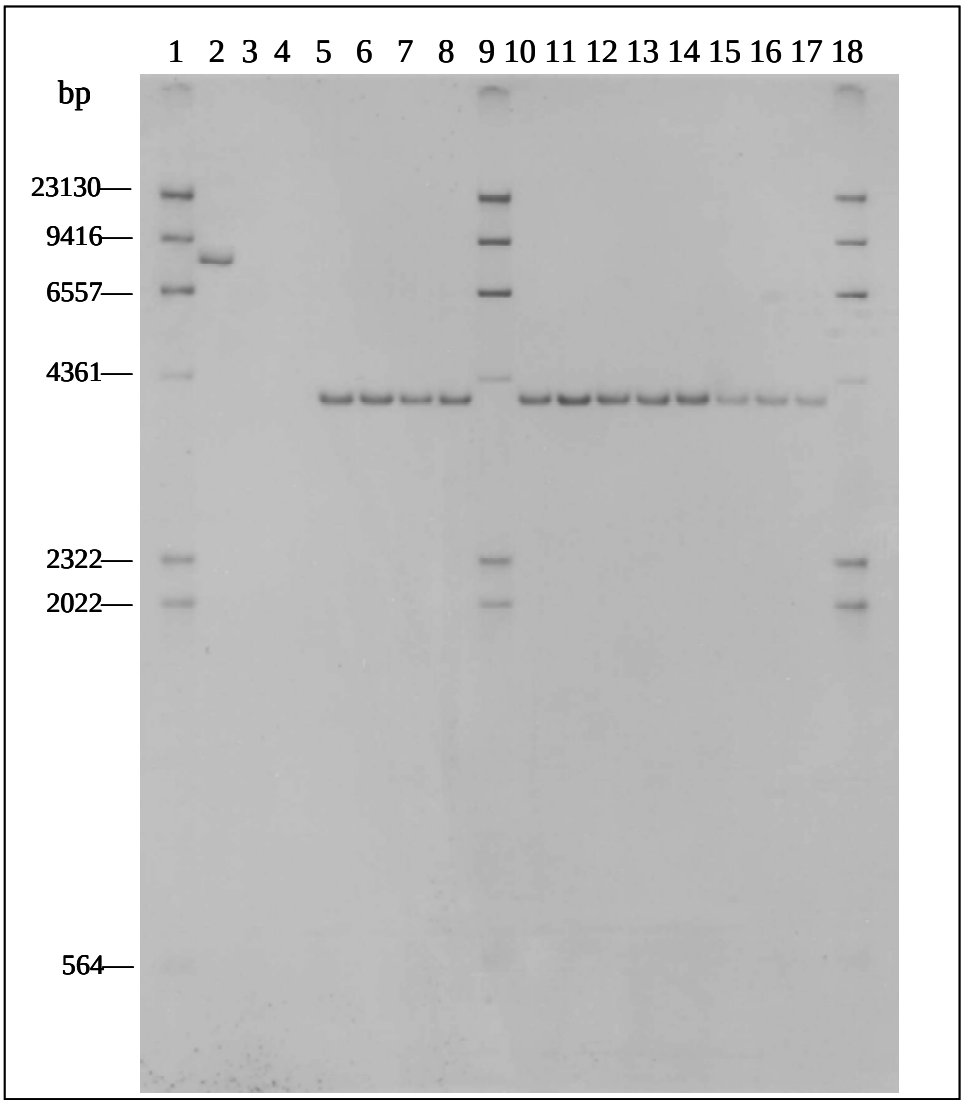
Figure 4. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Eco*R I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Eco*R I and probed with the *aad*-1 gene probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-4 | | 9 | DIG MWM II |
| 3 | XHH13-4 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

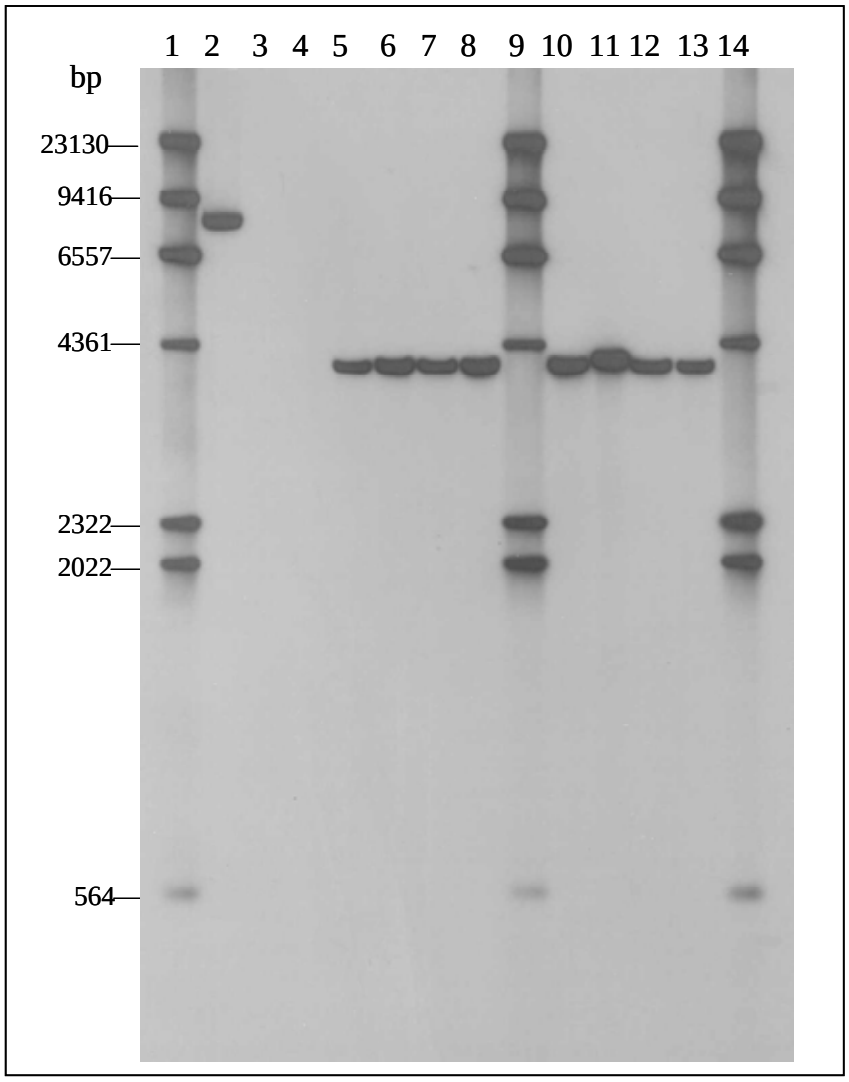
Figure 5. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Eco*R I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the *aad*-1 gene probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

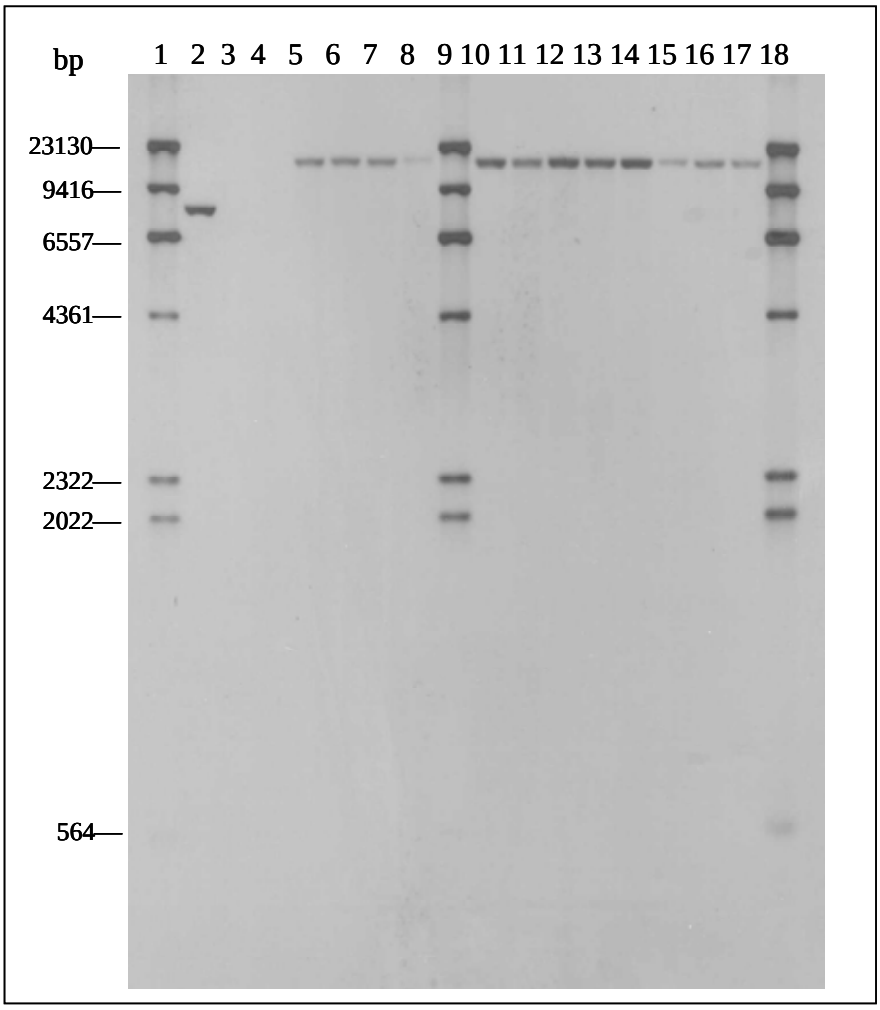
Figure 6. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the *aad*-1 gene probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-4 | | 9 | DIG MWM II |
| 3 | XHH13-4 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

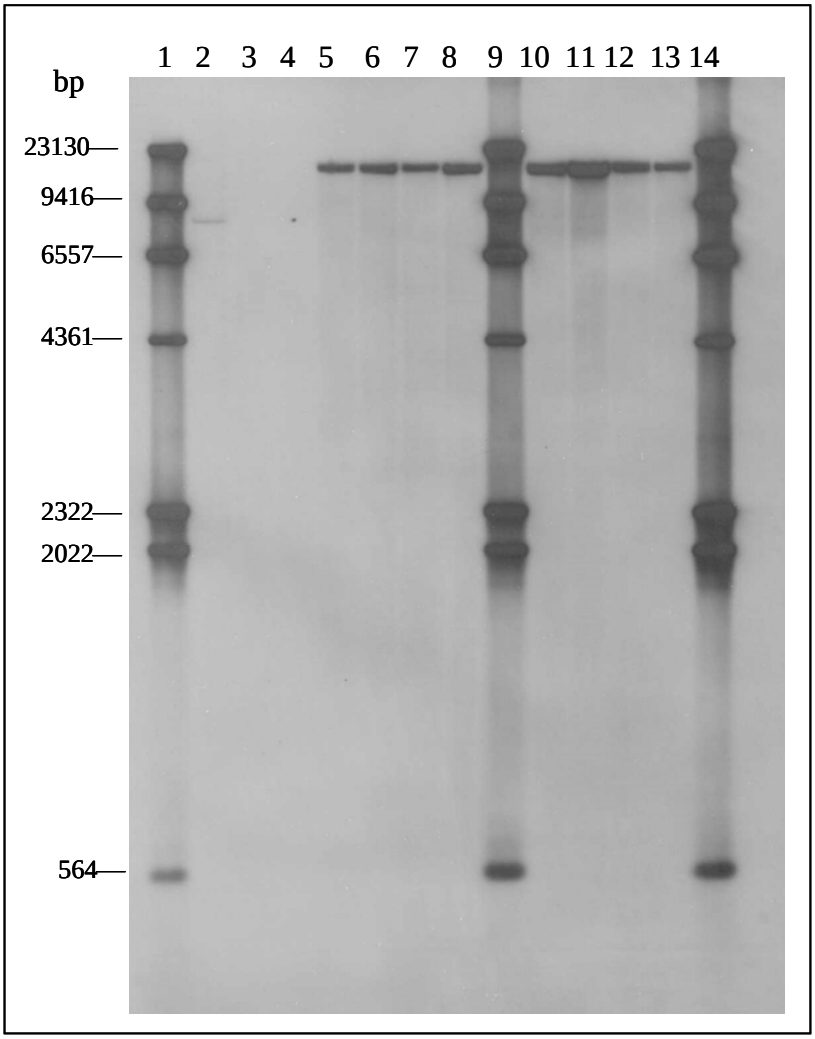
Figure 7. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the *aad*-1 gene probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note**: Weaker hybridization signal on lane 8 most likely due to DNA sample loss during the Southern hybridization process although same amount of genomic DNA was used in DNA digestion at the beginning of the process.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

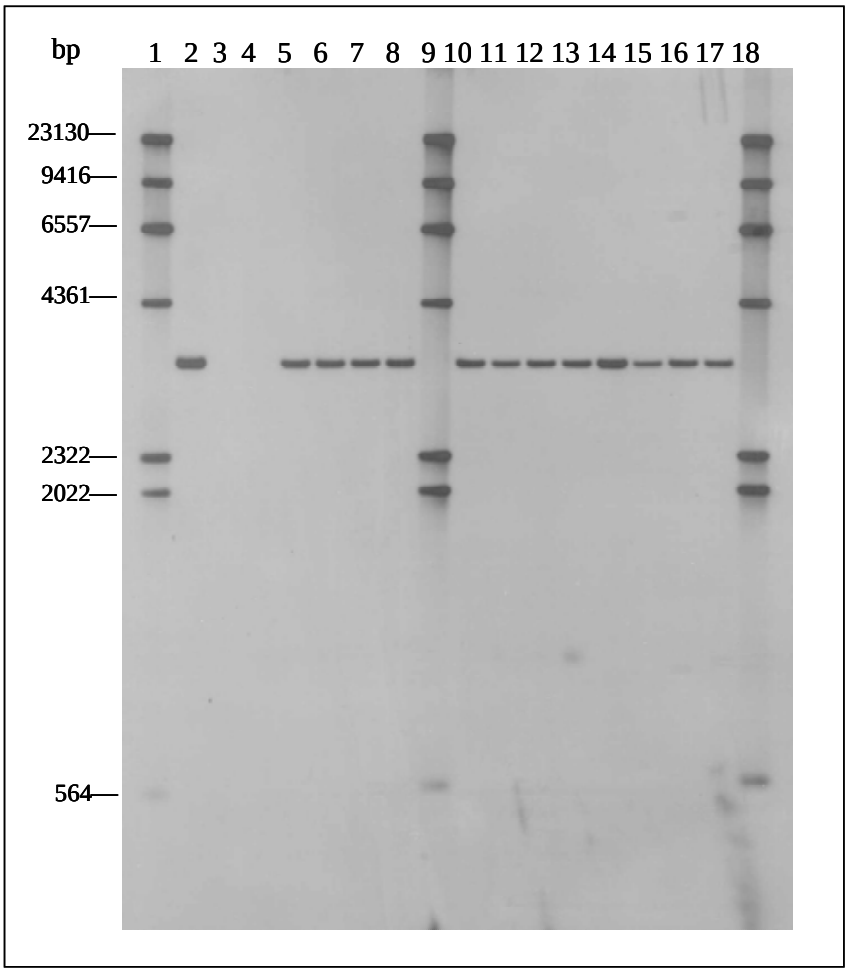
Figure 8. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the *aad*-1 gene probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-4 | | 9 | DIG MWM II |
| 3 | XHH13-4 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

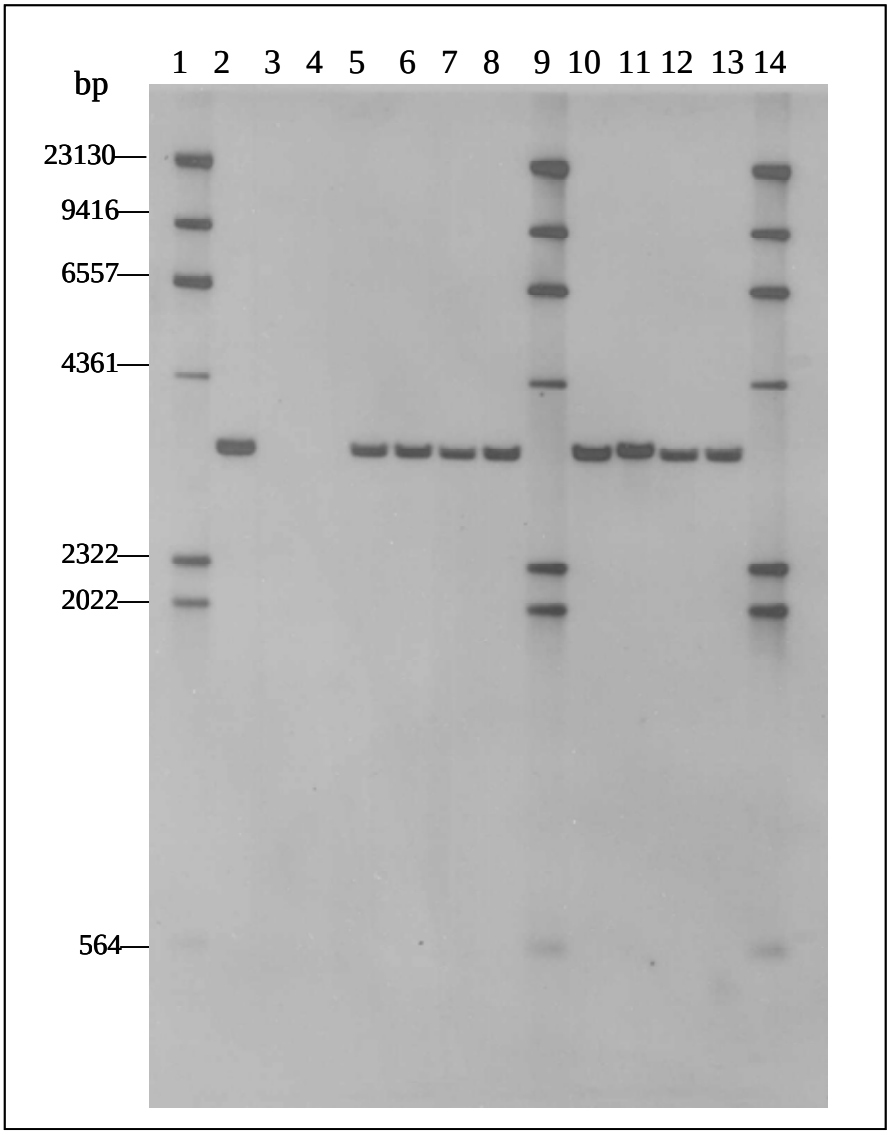
Figure 9. Southern blot analysis of DAS-40278-9; *aad-1* probe (OLP2), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Fse* I / *Hin*d III and probed with the *aad*-1 gene probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Background splotches are visible below the 2022bp marker between lanes11 and 18.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

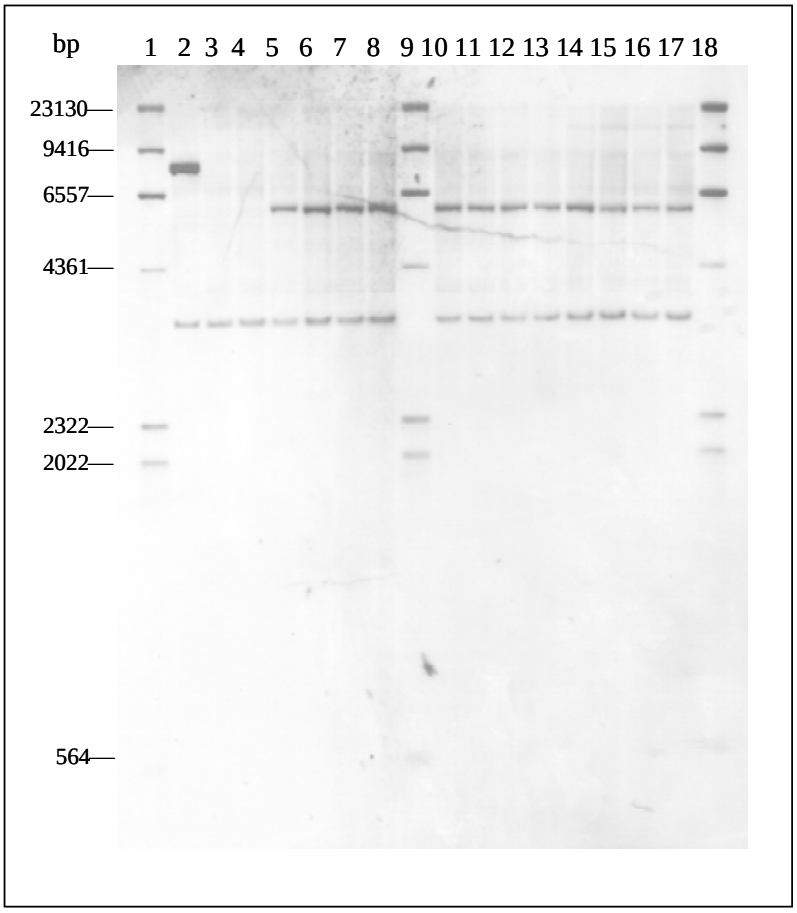
Figure 10. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Fse* I / *Hin*d III digest




Genomic DNA isolated from corn event DAS-40278-9 and unmodified corn XHH13 was digested with *Fse* I / *Hin*d III and probed with the *aad*-1 gene probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

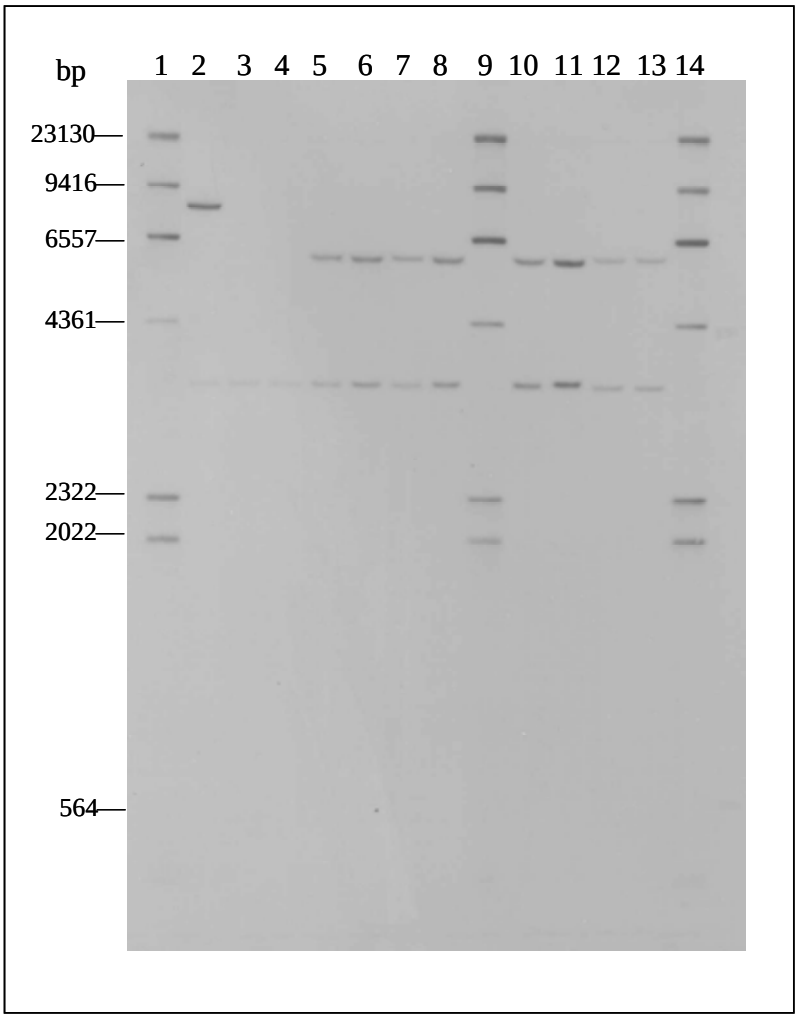
Figure 11. Southern blot analysis of DAS-40278-9; *aad*-1 probe (OLP2), *Fse* I / *Hin*d III digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the ZmUbi1 promoter probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous ubi gene in the corn genome at ~3600bp.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

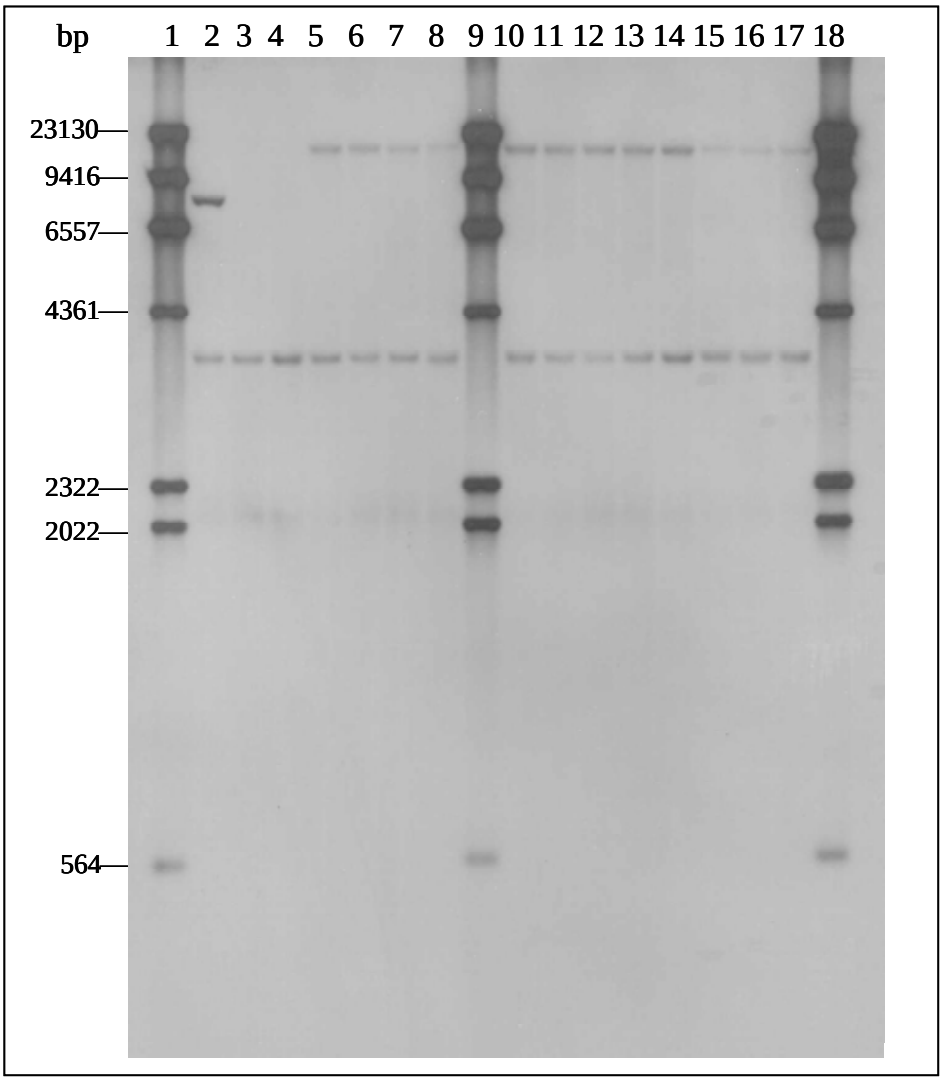
Figure 12. Southern blot analysis of DAS-40278-9; ZmUbi1 promoter probe (OLP1-3), Nco I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the ZmUbi1 promoter probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous ubi gene in the corn genome at ~3600bp.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

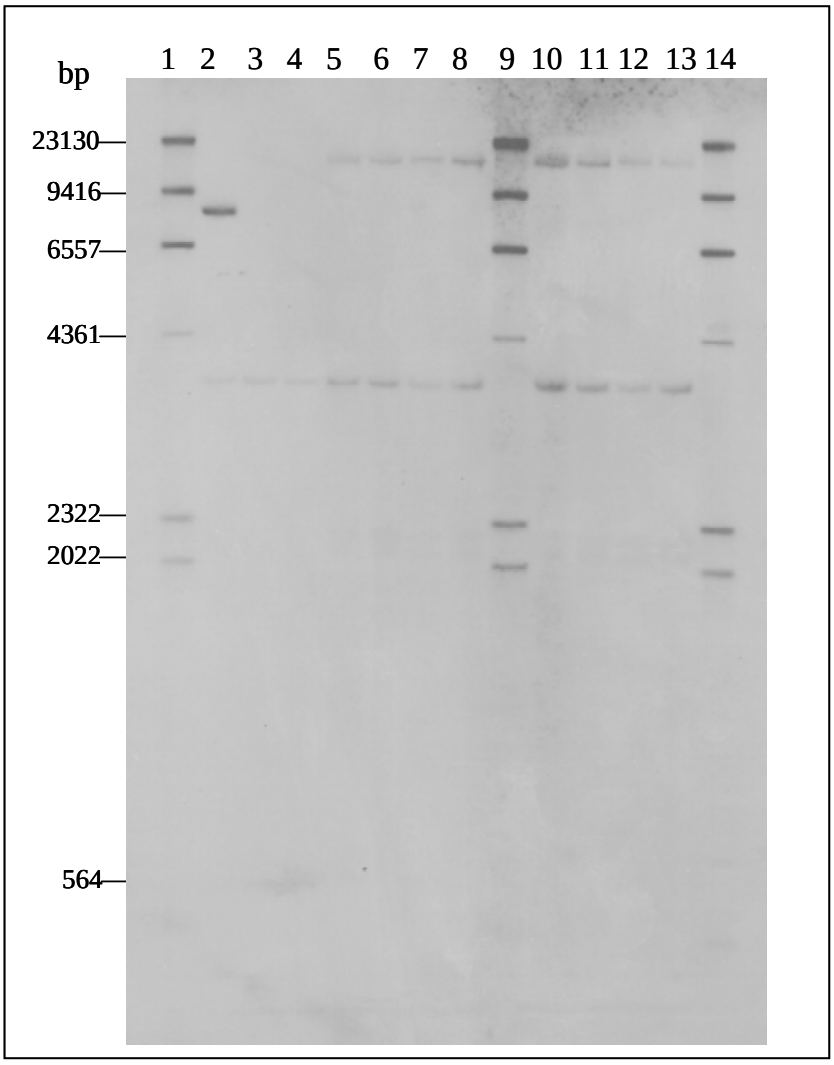
Figure 13. Southern blot analysis of DAS-40278-9; ZmUbi1 promoter probe (OLP1-3), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the ZmUbi1 promoter probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous ubi gene in the corn genome at ~3800bp. Background smears around 2022 bp marker across all sample lanes are lightly visible.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

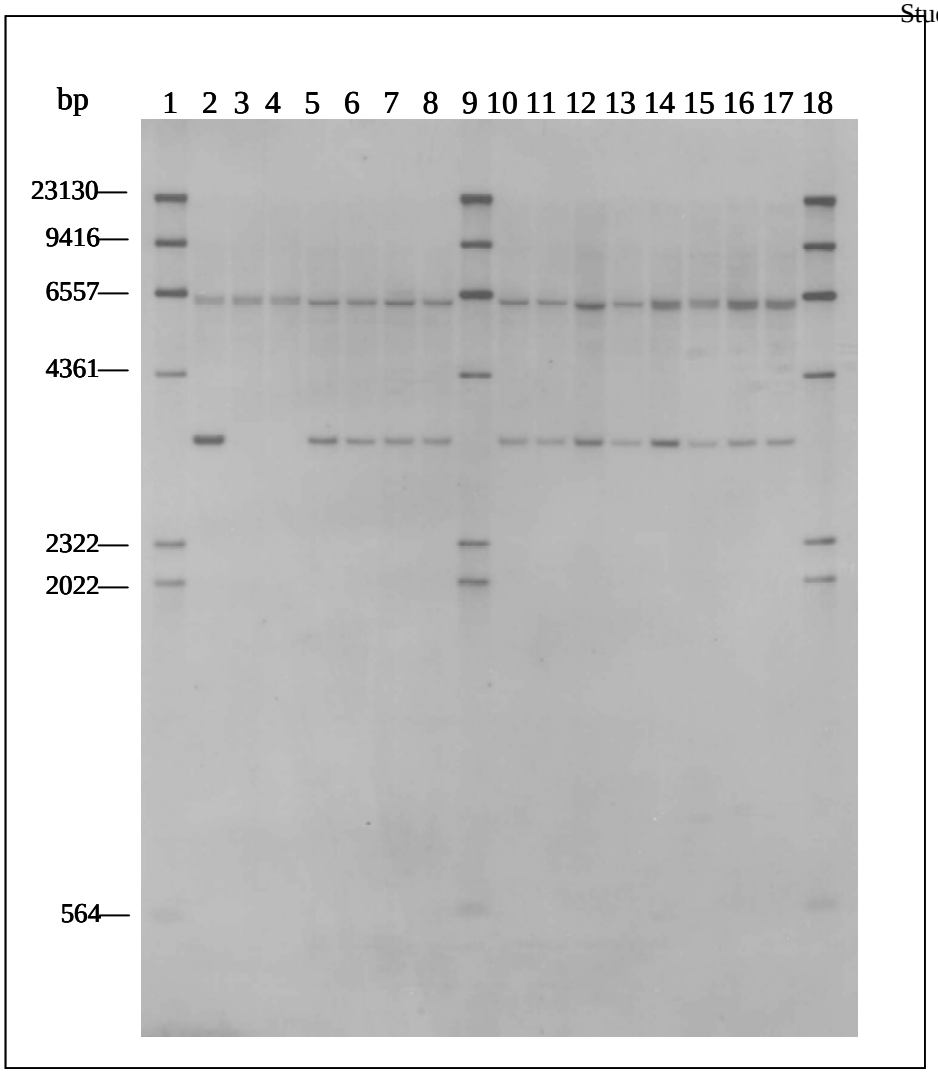
Figure 14. Southern blot analysis of DAS-40278-9; ZmUbi1 promoter probe (OLP1-3), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the ZmUbi1 promoter probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous ubi gene in the corn genome at ~3800bp.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

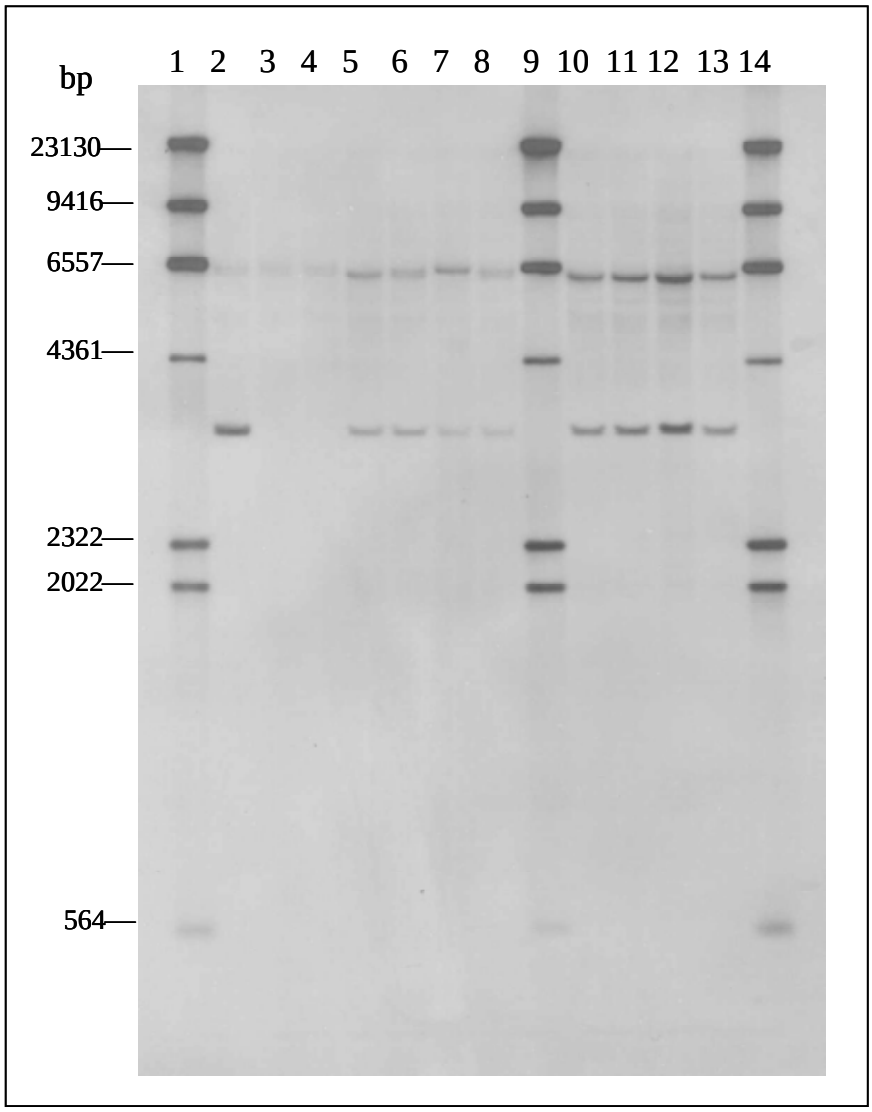
Figure 15. Southern blot analysis of DAS-40278-9; ZmUbi1 promoter probe (OLP1-3), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Fse* I / *Hin*d III and probed with the ZmUbi1 promoter probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous ubi gene in the corn genome at ~6400bp. Probe also hybridizes to another endogenous band most likely from the XHH13 genome at a position very close to ~6400bp in lanes 2-4 and lanes 14-17.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

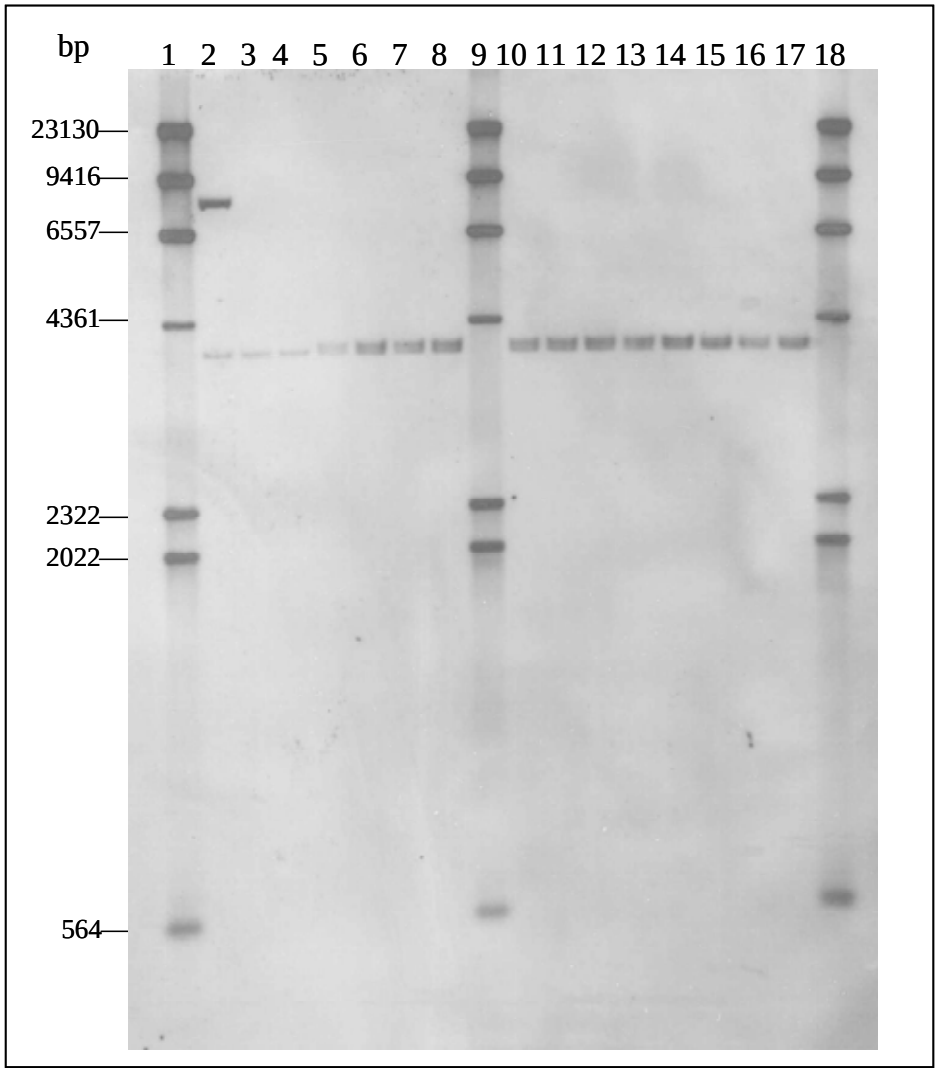
Figure 16. Southern blot analysis of DAS-40278-9; ZmUbi1 promoter probe (OLP1-3), *Fse* I / *Hin*d III digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Fse* I / *Hin*d III and probed with the ZmUbi1 promoter probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous ubi gene in the corn genome at ~6400bp. Probe also hybridizes to another endogenous band most likely from the XHH13 genome at a position very close to ~6400bp in lanes 2-4 and lanes 6 and 8.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

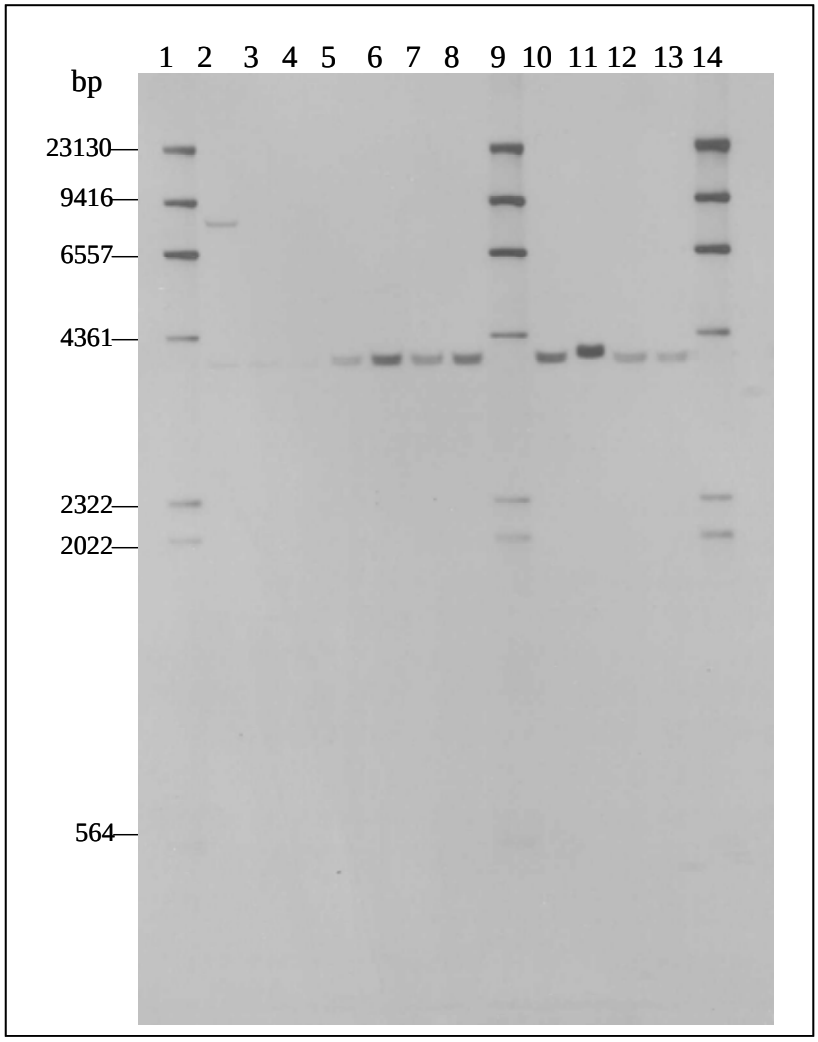
Figure 17. Southern blot analysis of DAS-40278-9; ZmUbi1 promoter probe (OLP1-3), *Fse* I / *Hin*d III digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the ZmPer5 terminator probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous per gene in the corn genome at ~3900bp.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

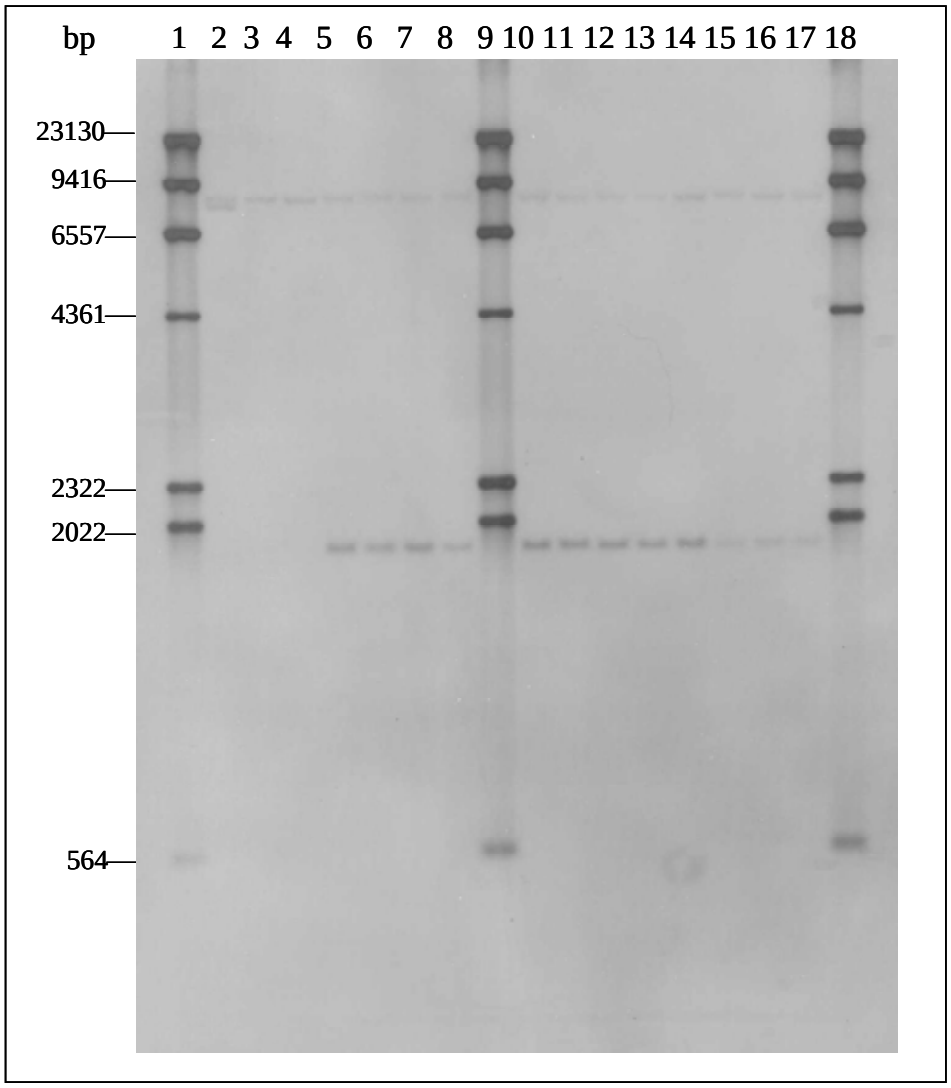
Figure 18. Southern blot analysis of DAS-40278-9; ZmPer5 terminator probe (OLP3A), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the ZmPer5 terminator probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous per gene in the corn genome at ~3900bp. Stronger signal and slightly slower migration of the hybridization bands in lane 11 are most likely due to more genomic DNA loaded although same amount of genomic DNA was used for digestion at the beginning of the process. Weaker endogenous hybridization band at ~3900 in lanes 2-4 are most likely due to less genomic DNA loaded although same amount of genomic DNA was used for digestion at the beginning of the process.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-4 | | 9 | DIG MWM II |
| 3 | XHH13-4 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

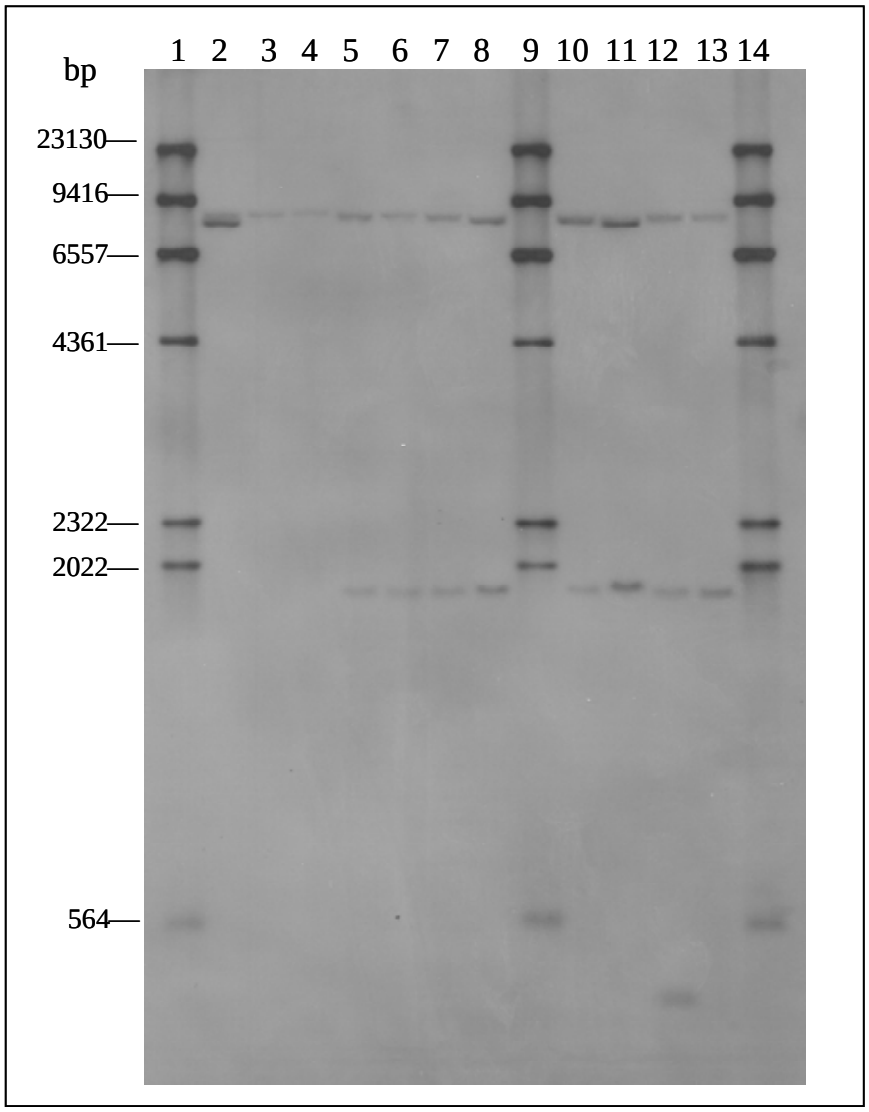
Figure 19. Southern blot analysis of DAS-40278-9; ZmPer5 terminator probe (OLP3A), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the ZmPer5 terminator probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous per gene in the corn genome at ~9000bp. Background splotch was visible around 564bp marker between lanes 13-14.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

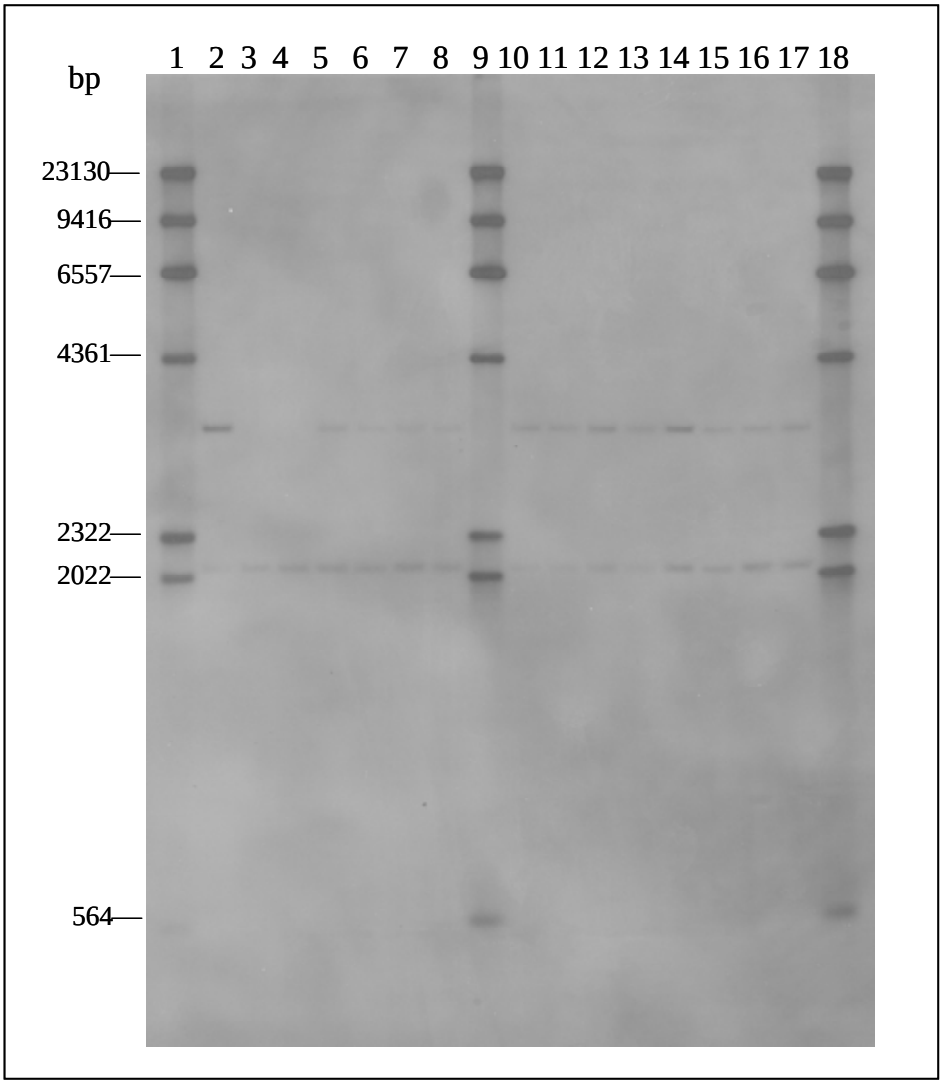
Figure 20. Southern blot analysis of DAS-40278-9; ZmPer5 terminator probe (OLP3A), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the ZmPer5 terminator probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous per gene in the corn genome at ~9000bp. Background splotch was visible below 564bp marker between lanes 11-12.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

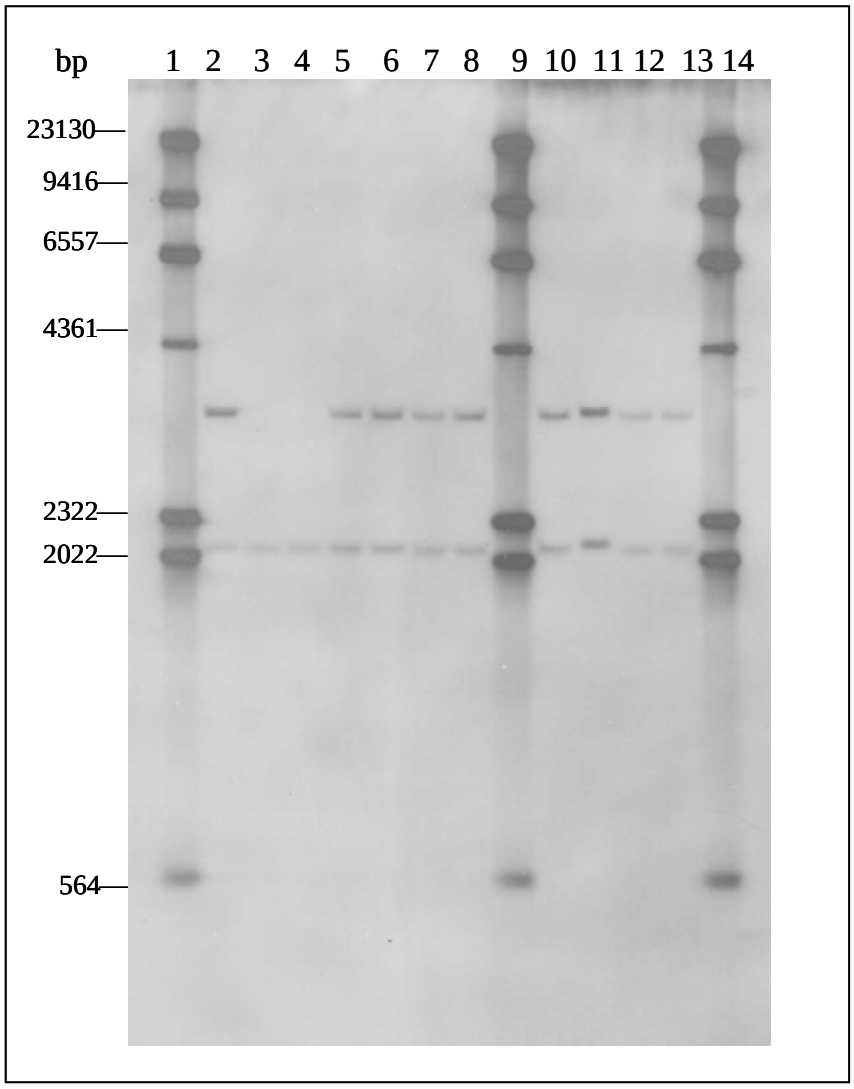
Figure 21. Southern blot analysis of DAS-40278-9; ZmPer5 terminator probe (OLP3A), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Fse* I / *Hin*d III and probed with the ZmPer5 terminator probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13. ***Note:*** *Probe hybridizes to endogenous per gene in the corn genome at ~2100bp.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-1 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-1 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

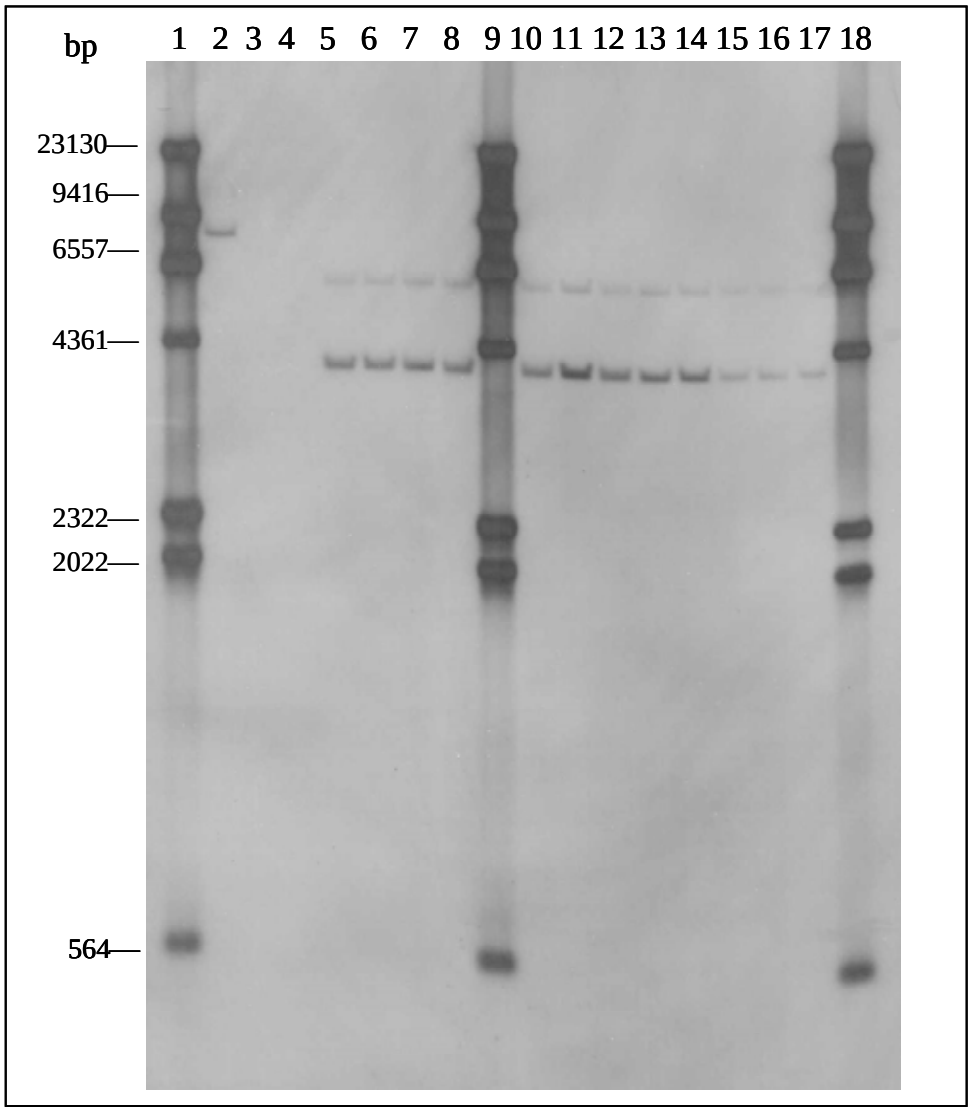
Figure 22. Southern blot analysis of DAS-40278-9; ZmPer5 terminator probe (OLP3A), *Fse* I / *Hin*d III digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Fse* I / *Hin*d III and probed with the ZmPer5 terminator probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13. . ***Note:*** *Probe hybridizes to endogenous per gene in the corn genome at ~2100bp.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

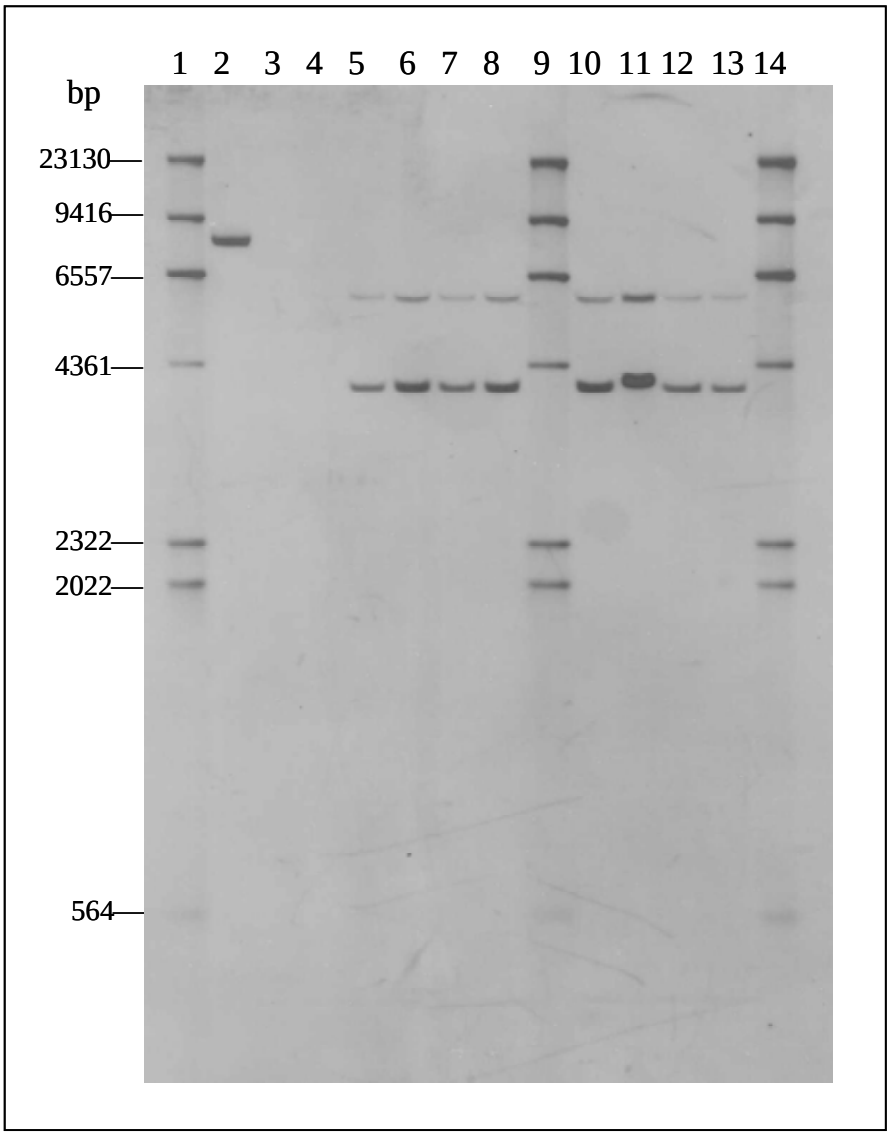
Figure 23. Southern blot analysis of DAS-40278-9; ZmPer5 terminator probe (OLP3A), *Fse* I / *Hin*d III digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the RB7 Mar v4 probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

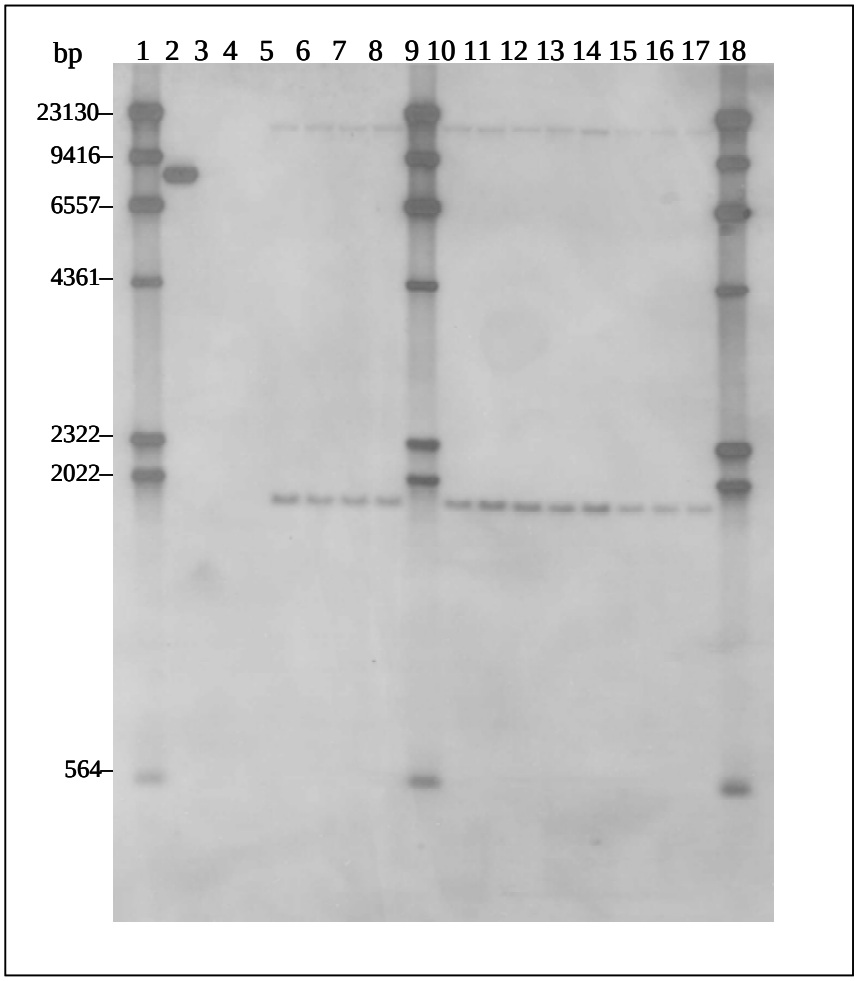
Figure 24. Southern blot analysis of DAS-40278-9; RB7 Mar v4 probe (OLP3B), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the RB7 Mar v4 probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-4 | | 9 | DIG MWM II |
| 3 | XHH13-4 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

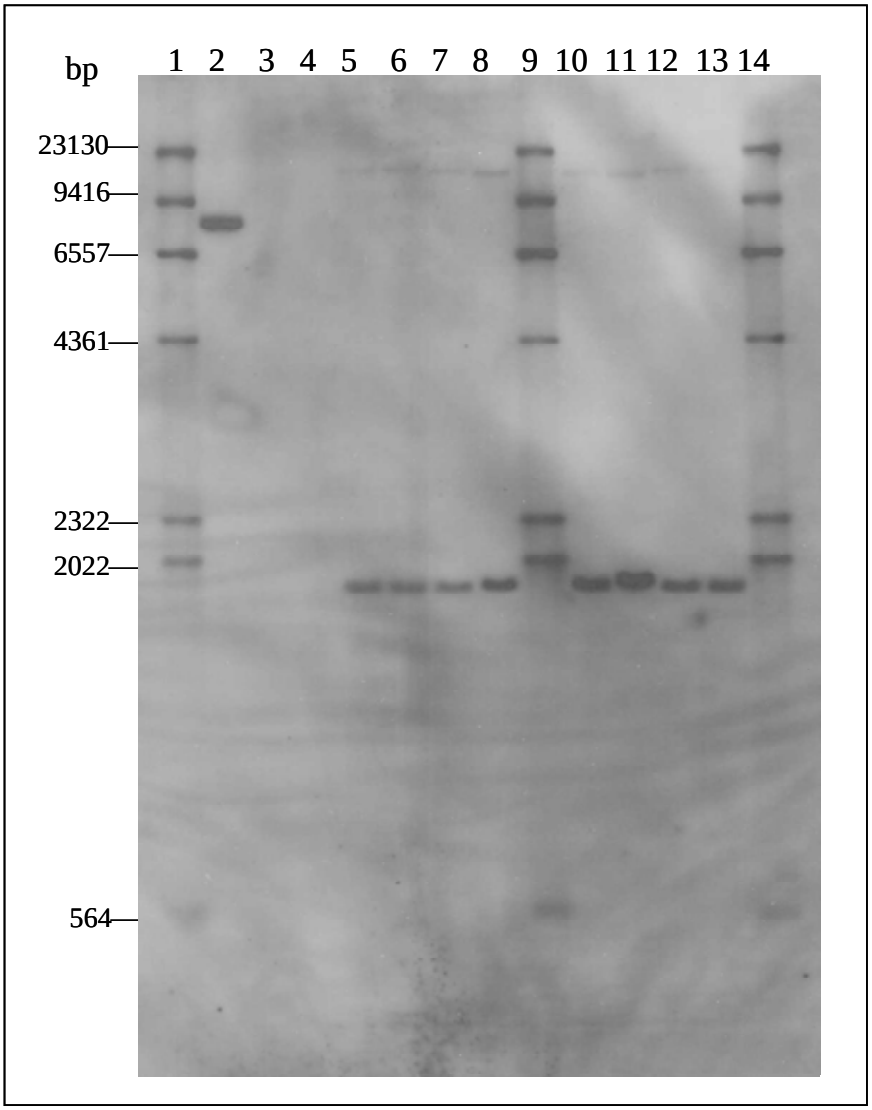
Figure 25. Southern blot analysis of DAS-40278-9; RB7 Mar v4 probe (OLP3B), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the RB7 Mar v4 probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

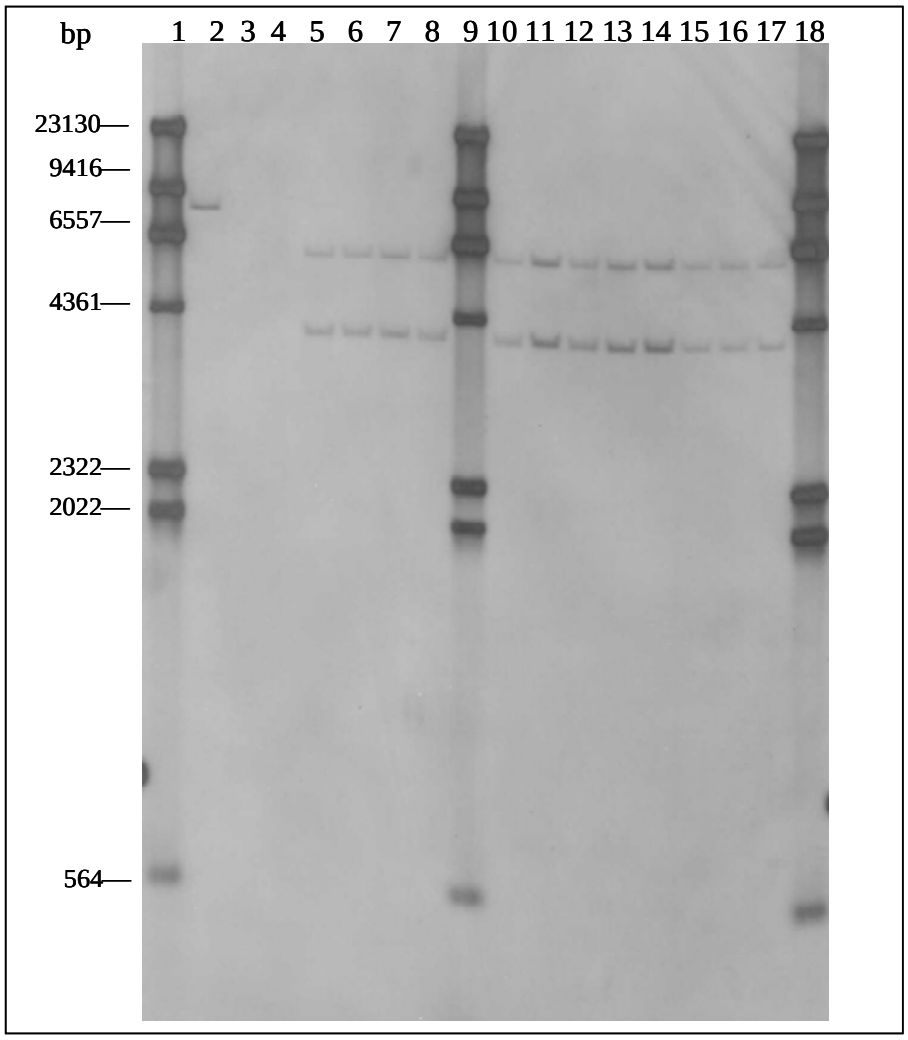
Figure 26. Southern blot analysis of DAS-40278-9; RB7 Mar v4 probe (OLP3B), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the RB7 Mar v4 probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

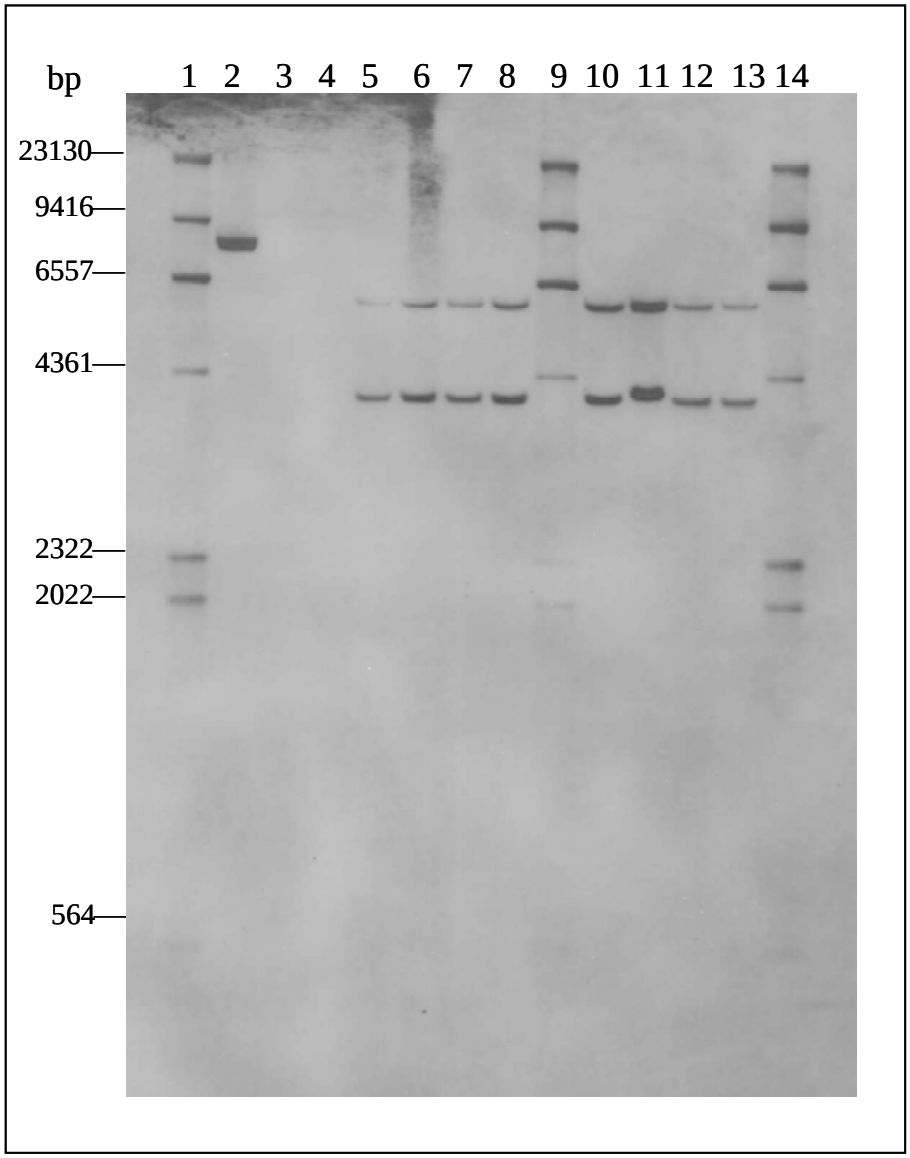
Figure 27. Southern blot analysis of DAS-40278-9; RB7 Mar v4 probe (OLP3B), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the RB7 Mar v3 probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

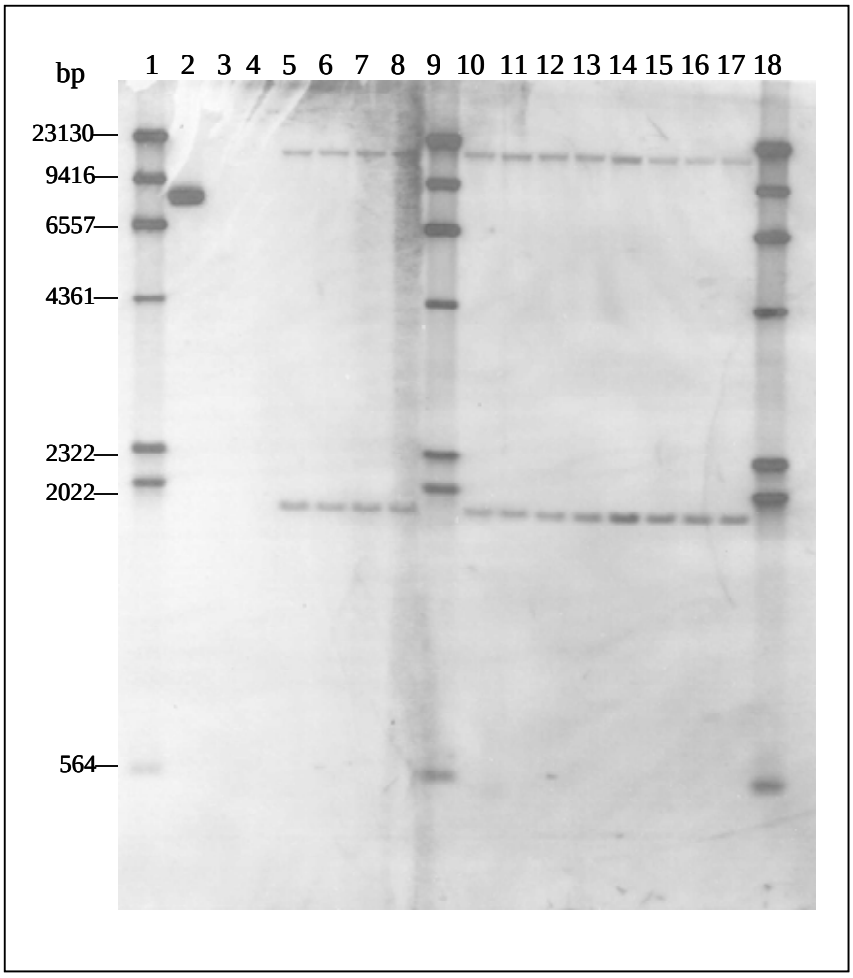
Figure 28. Southern blot analysis of DAS-40278-9; RB7 Mar v3 probe (OLP5-2), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the RB7 Mar v3 probe. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-4 | | 9 | DIG MWM II |
| 3 | XHH13-4 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

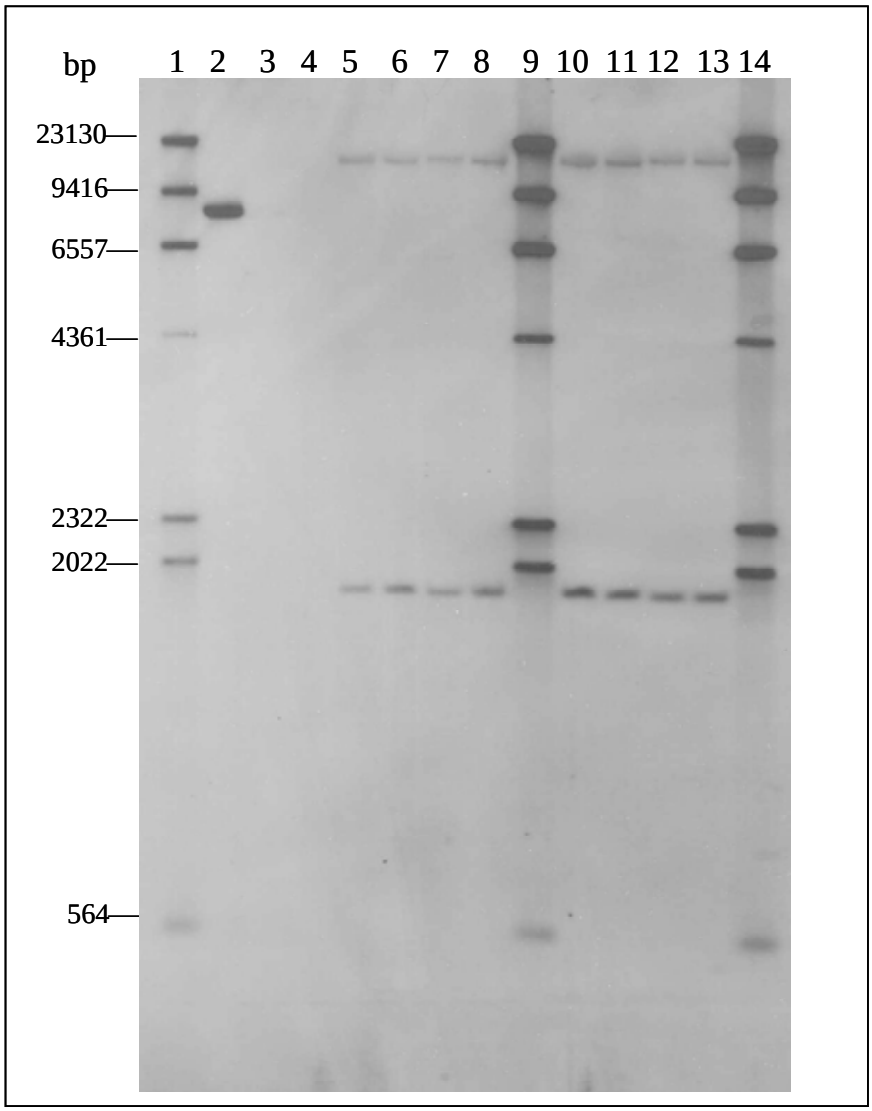
Figure 29. Southern blot analysis of DAS-40278-9; RB7 Mar v3 probe (OLP5-2), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the RB7 Mar v3 probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note**: Background splotches were visible above 23130bp marker between lanes 2-11 and almost across the blot for lane 8.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

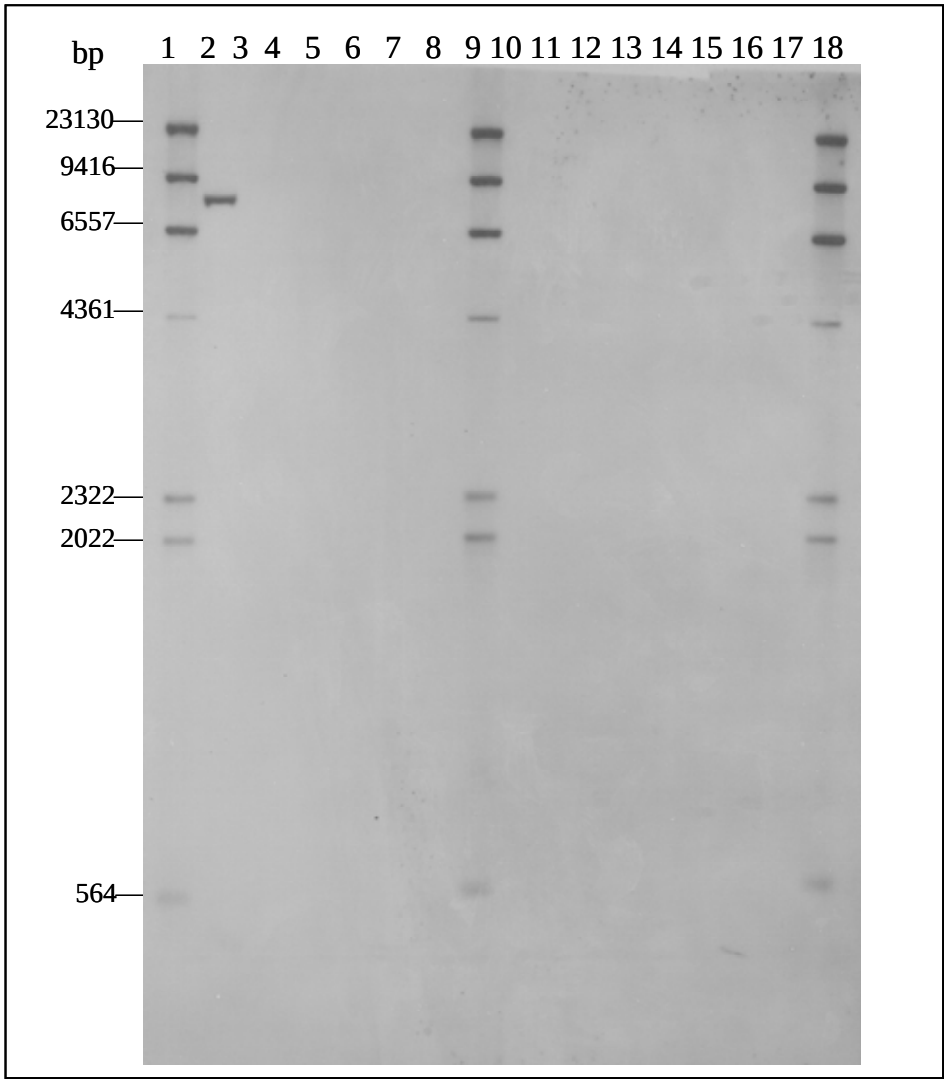
Figure 30. Southern blot analysis of DAS-40278-9; RB7 Mar v3 probe (OLP5-2), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the RB7 Mar v3 probe. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

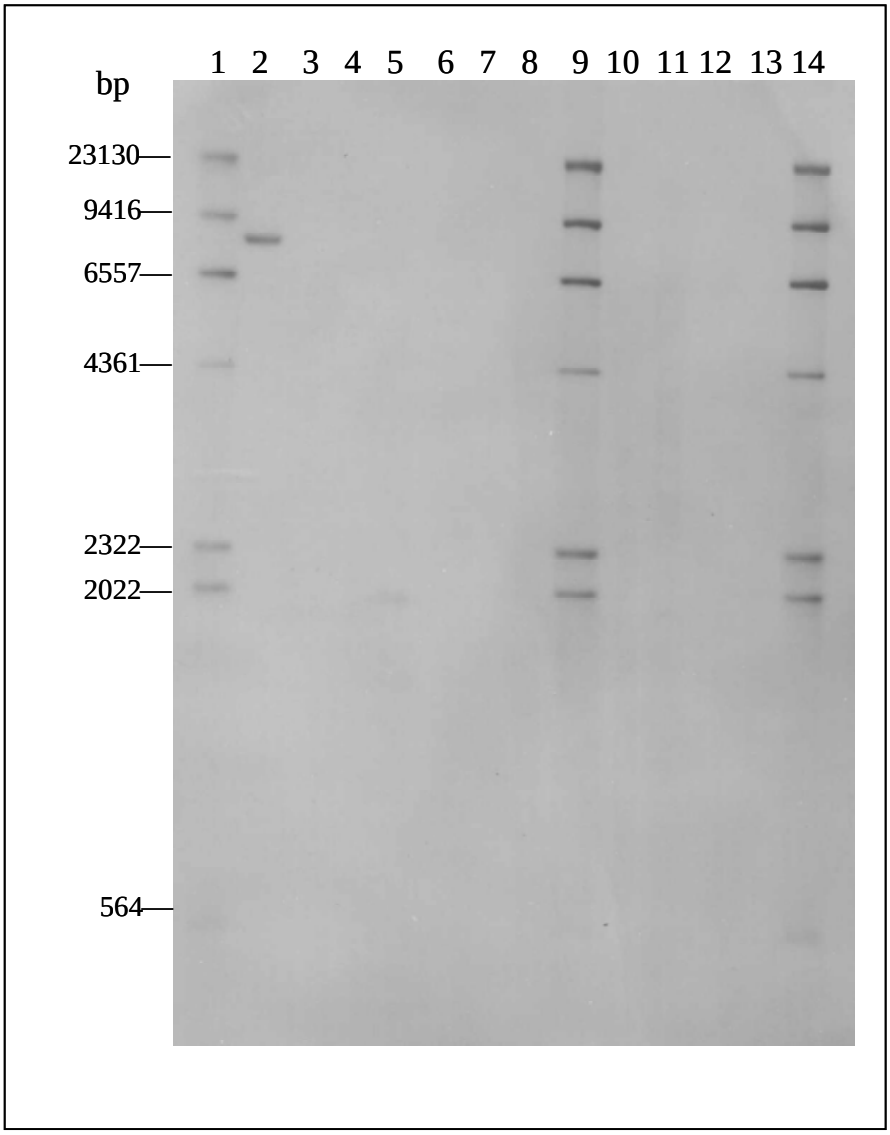
Figure 31. Southern blot analysis of DAS-40278-9; RB7 Mar v3 probe (OLP5-2), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the backbone probes OLP4ABC. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

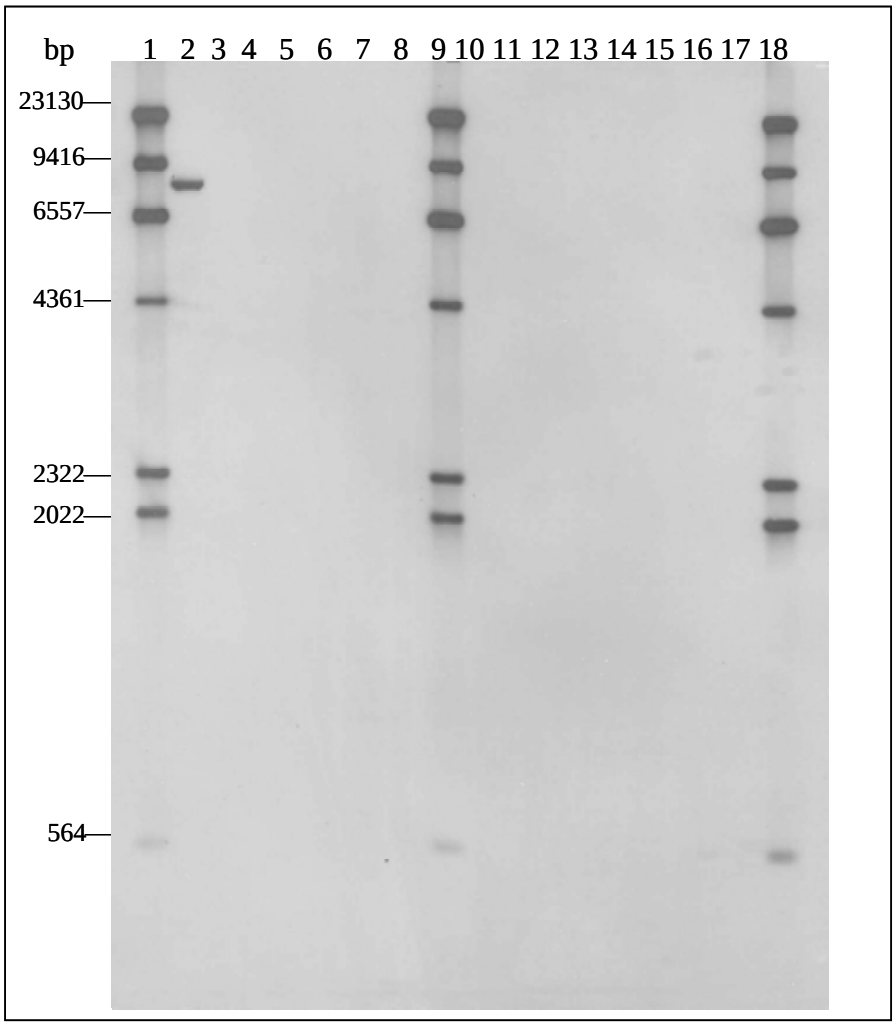
Figure 32. Southern blot analysis of DAS-40278-9; backbone probes (OLP4ABC), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Nco* I and probed with the backbone probes OLP4ABC. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13. ***Note****: Background splotch was visible around 2022bp marker between lanes 4 and 5.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

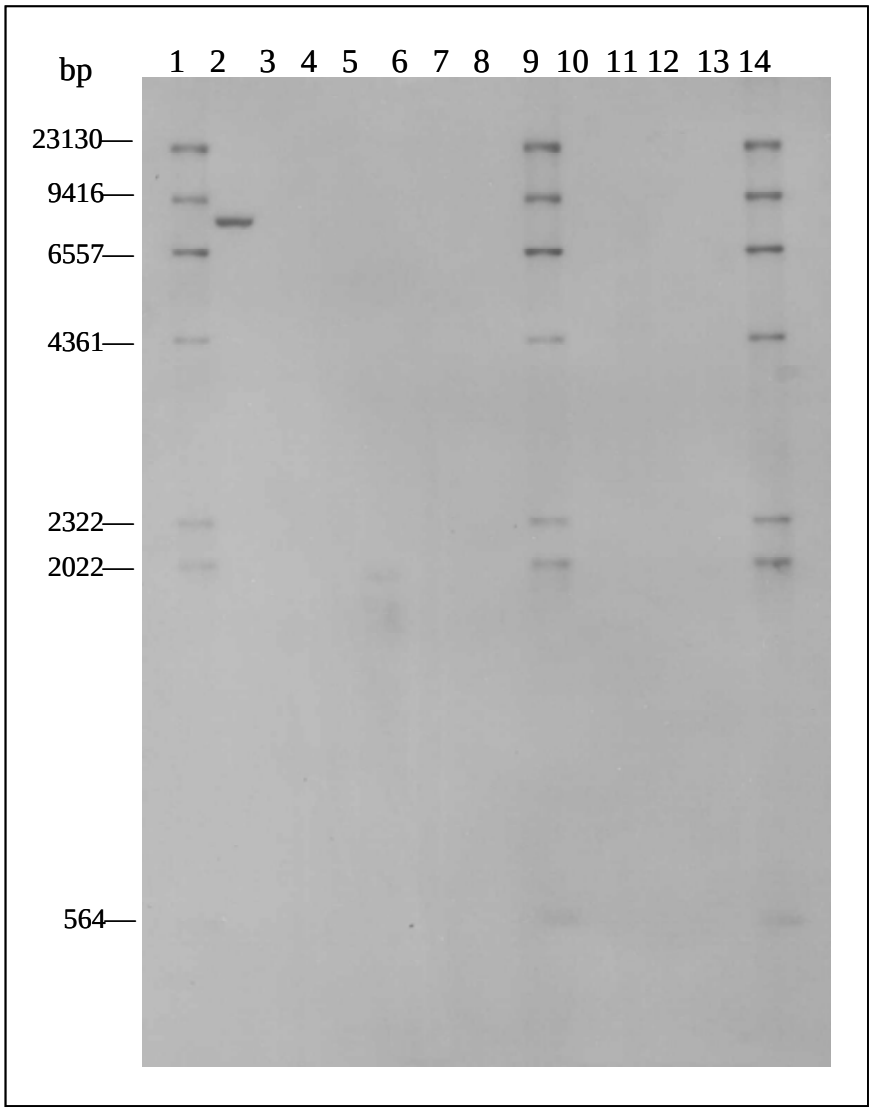
Figure 33. Southern blot analysis of DAS-40278-9; backbone probes (OLP4ABC), *Nco* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the backbone probes OLP4ABC. Nine (9) μg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 μg of genomic DNA isolated from the conventional control XHH13.

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 10 | DAS40278-9-T4-1 |
| 2 | pDAS1740 + XHH13-4 | | 11 | DAS40278-9-T4-3 |
| 3 | XHH13-4 | | 12 | DAS40278-9-T4-5 |
| 4 | XHH13-2 | | 13 | DAS40278-9-T4-6 |
| 5 | DAS40278-9-T3-1 | | 14 | DAS40278-9-BC3S1-6 |
| 6 | DAS40278-9-T3-4 | | 15 | DAS40278-9-BC3S1-8 |
| 7 | DAS40278-9-T3-13 | | 16 | DAS40278-9-BC3S1-11 |
| 8 | DAS40278-9-T3-14 | | 17 | DAS40278-9-BC3S1-12 |
| 9 | DIG MWM II | | 18 | DIG MWM II |

Figure 34. Southern blot analysis of DAS-40278-9; backbone probes (OLP4ABC), *Sac* I digest




Genomic DNA isolated from corn event DAS-40278-9 and conventional corn XHH13 was digested with *Sac* I and probed with the backbone probes OLP4ABC. Nine (9) µg of DNA were digested and loaded per lane. The plasmid control contained the approximate equivalent of 1 transgene copy per genome of plasmid pDAS1740 and 9 µg of genomic DNA isolated from the conventional control XHH13. ***Note**: Background splotches were visible below 2022bp marker between lanes 5 and 6.*

| Lane | Sample | | Lane | Sample |
|---|---|---|---|---|
| 1 | DIG MWM II | | 8 | DAS40278-9-BC3S2-5 |
| 2 | pDAS1740 + XHH13-5 | | 9 | DIG MWM II |
| 3 | XHH13-5 | | 10 | DAS40278-9-BC3S3-2 |
| 4 | XHH13-2 | | 11 | DAS40278-9-BC3S3-3 |
| 5 | DAS40278-9-BC3S2-1 | | 12 | DAS40278-9-BC3S3-5 |
| 6 | DAS40278-9-BC3S2-2 | | 13 | DAS40278-9-BC3S3-7 |
| 7 | DAS40278-9-BC3S2-3 | | 14 | DIG MWM II |

Figure 35. Southern blot analysis of DAS-40278-9; backbone probes (OLP4ABC), *Sac* I digest