Title

Structural stability analysis of *Glycine max* event FG72 in different generations, in different backgrounds and when grown in different environments

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Author

Steven Verhaeghe

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Testing Facility

Bayer BioScience N.V.
BioAnalytics
Molecular Characterization
Technologiepark 38
B-9052 Gent
Belgium

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Test Facility Address:	Bayer BioScience N.V. BioAnalytics - Molecular Characterization Technologiepark 38 9052 Ghent – Belgium Tel: +32 9-243 04 11 Fax: +32 9-224 06 94
Molecular Characterization Manager:	Dirk Nennstiel Address see Test Facility Tel: +32 9-243 04 39 Fax: +32 9-224 06 94 e-mail: dirk.nennstiel@bayercropscience.com
Study Manager/Study Director:	Steven Verhaeghe Address see Test Facility Tel: +32 9-243 05 55 Fax: +32 9-224 06 94 e-mail: steven.verhaeghe@bayercropscience.com
Study Personnel	Tommy Vansteenkiste Nele Vandermarliere Marianne Van De Wiele Address see Test Facility
Sponsor:	Regulatory Affairs
Sponsor representative:	Russell Essner Global Regulatory Affairs Manager P.O. Box 12014 2 T.W. Alexander Drive Research Triangle Park, NC 27709 – United States Tel: +1 919 549 2171 Fax: +1 919 549 2892 e-mail: russ.essner@bayercropscience.com

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SUMMARY

The *Glycine max* transformation event FG72 was produced by means of direct gene transfer of soybean cells using a HPLC purified 7.3kb *Sal*I fragment of vector pSF10, containing a *2mepsps* gene construct, encoding tolerance to glyphosate, and a *hppdPf W336* gene construct, encoding tolerance to isoxaflutole. *Glycine max* transformation event FG72 contains two partial 3'histonAt sequences in a head to head orientation, followed by 2 complete T-DNA copies arranged in a head to tail orientation. Upon integration of the FG72 insert into the *Glycine max* genome, a genomic region translocated to a new position, which is joined by 158 bases of Ph4a748 promoter sequences (Verhaeghe S., 2010).

To demonstrate the structural stability of *Glycine max* transformation event FG72, genomic DNA was prepared from several individual plants of three generations and three different genetic backgrounds. The impact of environment was assessed by analyzing the progeny of transgenic plants cultivated at 4 different field locations. The isolated DNA was digested with the restriction enzyme *Hin*dIII, which provides a unique pattern for transformation event FG72.

Digested genomic FG72 DNA was probed with both the Ph4a748B probe and the complete T-DNA probe. The complete T-DNA probe revealed the FG72 internal fragments and the 5' and the 3' integration fragments. The 158 bases of Ph4a748 promoter sequences located next to the translocated sequence could not always be visualized using the T-DNA probe. The stability of this region was demonstrated using the Ph4a748B probe. The obtained Southern blot profile was identical for all samples, which demonstrates the stability of transformation event FG72 at the genomic level in different generations, different environments and different backgrounds.

1. OBJECTIVE

The *Glycine max* transformation event FG72 was produced by means of direct gene transfer of soybean cells using a HPLC purified 7.3kb *Sal*I fragment of vector pSF10, containing a *2mepsps* gene construct, encoding tolerance to glyphosate, and a *hppdPf W336* gene construct, encoding tolerance to isoxaflutole. *Glycine max* transformation event FG72 contains two partial 3'histonAt sequences in a head to head orientation, followed by two complete T-DNA copies arranged in a head to tail orientation. Upon integration of the FG72 insert into the *Glycine max* genome, a genomic region translocated to a new position, which is joined by 158 bases of Ph4a748 promoter sequences (Verhaeghe S., 2010).

In this study the structural stability of the *Glycine max* event FG72 was demonstrated by testing leaf tissue from plants of different generations, different backgrounds and the progeny of plants grown in different environments by means of Southern blot analysis.

2. TEST ITEMS

Seeds of *Glycine max* transformation event FG72 harvested in different locations, of different generations, and of different genetic backgrounds (Table 1) were sown in the Bayer BioScience N.V. greenhouse (Astene, Belgium). For each obtained FG72 plant, the FG72 identity and zygosity was confirmed by means of zPCR (protocol MDP0726_01). Leaf tissue harvested from these different individual plants was used as the test item in this study. Harvested tissues were directly frozen in liquid nitrogen and stored in the ultrafreezer until DNA preparation. Leaf tissue from non-transgenic plants of variety Jack was prepared under identical conditions as the test item. This non-transgenic leaf material was used as the reference item. The pSF10 plasmid DNA (Figure 1) was the control item.

Table 1: Overview of the tested seed lots of FG72

Different locations for seed lot production	Generation	Seed lot n°
Adel, Iowa	T9	HT08SOY002-05-32
Osborn, Missouri	T9	HT08SOY002-07-32
Fithian, Illinois	T9	HT08SOY002-08-32
Sharpsville, Indiana	T9	HT08SOY002-09-32

Different generations	Generation	Seed lot n°
T2	T2	FG72a-T2, FG72b-T2, FG72c-T2, FG72d-T2 **
T7	T7	FG72-x-x-14-5-1-6-x T7
T9	T9	All seed lots of different locations

Different backgrounds	Generation	Seed lot n°
3068115-48 x Jack *	F4	7BD60018
3066617-48 x Jack *	F4	7BD60008
Jack		All seed lots of different locations and generations tested

* Conventional lines 3068115-48 and 3066617-48 were crossed with FG72 in Jack genetic background one time and then selfed 3 times.

** Seeds lots FG72a-T2, FG72b-T2, FG72c-T2 and FG72d-T2 are each the progeny of 1 individual seed of FG72 T1 generation. Leaf material grown from these T2 seeds was randomly collected from individual plants and used in this study as T2 generation.

3. SOUTHERN BLOT ANALYSIS

3.1 Genomic DNA preparation

Total genomic DNA was isolated from leaf tissues harvested from individual transgenic plants and from non-transgenic plants according to Dellaporta *et al.* (1983). The DNA quality was checked by loading a dilution on a 1% TAE gel.

3.2 Restriction digests of total genomic DNA and of plasmid DNA

Each prepared genomic DNA sample and the transforming plasmid DNA were digested with *Hin*dIII restriction enzyme. The digestions were stored frozen.

3.3 Sample preparation

The concentration of each digested genomic DNA sample and of the digested pSF10 plasmid DNA was determined by means of PicoGreen® measurements. Equal amounts of each digested genomic DNA preparation were supplemented with gel loading dye before loading on the agarose gel. The negative control was genomic DNA prepared from non-transgenic leaf material that was digested with *Hin*dIII. The positive control was genomic DNA prepared from non-transgenic leaf material that was digested with *Hin*dIII and supplemented with an equimolar amount of *Hin*dIII digested pSF10 plasmid.

3.4 Agarose gel electrophoresis

For each seed lot, one 1% TAE agarose gel was prepared. All digested FG72 genomic DNA samples, the positive and the negative control, and phage Lambda DNA digested with *Pst*I and phage Lambda DNA digested with *Hin*dIII as molecular weight markers were loaded on the agarose gel. The gel was run at 40 V until the bromophenol blue dye had migrated approximately 13 cm (approximately 18 hours).

After completion of the agarose gel electrophoresis, the gel was transferred to the UV transilluminator and a phosphorescent ruler was placed along the side of the gel where the molecular weight markers were loaded. A photograph of the gel was taken and printed using the Genius:BOX BioImaging system (G:BOX) (Syngene).

3.5 Blotting of the restriction fragments

After electrophoresis, the gels were transferred to a glass tray and depurinated with a 0.25 M HCl solution in MQ water and gently shaken until the bromophenol blue, deriving from the loading dye, turned yellow (approximately 20 minutes). The solution was removed and the gels were rinsed with distilled water.

The gels were then soaked in several gel volumes of denaturation solution (1.5 M NaCl and 0.5 M NaOH in MQ water) while smoothly shaking for 30 minutes. The denaturation solution was removed and rinsed two times with distilled water. This denaturation step was performed two times.

The gels were then subsequently soaked in several volumes of neutralisation solution (1.5M NaCl, 1M Tris-base, pH 7.4) while gently shaken. The neutralisation solution was removed and the gels were rinsed two times with distilled water. This neutralisation step was performed two times before blotting.

A Pyrex oven dish of 25 x 25 cm was filled with 20xSSC. A platform was made by placing a 20 x 40 cm glass plate on the oven dish. A piece of Whatman 3MM filter paper (used as wick) was cut at the exact width of the gel. The wick was placed on the glass plate and saturated with 20xSSC. Next, the gel was placed on the Whatman 3MM filter paper (wick). Care was taken to avoid trapping air bubbles underneath the gel.

A sheet of Hybond-N^+ membrane (GE Healthcare) was cut to the exact size of the gel. The membrane was wetted with MQ water and placed on top of the gel. Using a clean glass pipette as a rolling pin, all air bubbles beneath the membrane were rolled out. One sheet of Whatman 3MM paper was cut to size and wetted with MQ water and was placed on top of the Hybond-N^+ membrane. The gel was surrounded with plastic wrap to prevent the 20x SSC being absorbed directly into the paper towels above. A stack of absorbent paper towels was placed on top of the 3MM paper. Finally, a glass plate was placed on top of the paper towels along with a 0.5 to 1 kg weight. Transfer of the DNA to the Nylon membrane was allowed to proceed for 12 to 48 hours.

After blotting, the set-up was carefully disassembled. Before removing the gel from the membrane, the position of the wells, as well as the code of the gel, was marked on the membrane with a pencil. The membrane was transferred to a Whatman 3MM filter paper and air dried for 10 minutes. The membrane was then baked for at least 2 hours at 80°C. The blot was stored between sheets of Whatman 3MM paper in a clean dry place at room temperature until hybridization.

3.6 Probe template preparation

Two probes were used to assess the stability of FG72: a complete 'T-DNA probe' and a 'Ph4a748B probe'. Information on the probe templates and on the primers and restriction enzymes used to prepare the probe templates is shown in Table 2 and Table 3.

The T-DNA probe template was prepared by means of a *Sac*I/*Sma*I restriction digestion of the pSF10 plasmid DNA, according to the conditions proposed by the manufacturer. To purify the probe template, the complete reaction mixture of the restriction digestion was loaded on a 1% TAE agarose gel, the fragment of 7204 bp was cut out of the gel and the probe template was isolated from the gel slice using the QIAquick Gel Extraction Kit (QIAgen).

The Ph4a748B probe template was synthesized by means of PCR amplification using the Expand™ High Fidelity PCR system and pSF10 plasmid DNA as template DNA. Since both primers have two priming sites in plasmid pSF10 (Table 3), two amplicons were obtained. After loading the complete reaction mixture on a 1% TAE agarose gel, the 430 bp amplicon was cut out of the gel and the probe template was isolated from the gel slice using the QIAquick Gel Extraction Kit (QIAgen).

The concentration of the recovered fragments was estimated by loading a small amount of the sample on a 1% agarose gel and comparing band intensities with a known standard (High or Low DNA mass ladder from Invitrogen for T-DNA probe and Ph4a748B probe respectively).

3.7 Labelling of the probes

Forty nanograms of the probe template was labelled with [α-^{32}P]-dCTP (10 mCi/mL at Activity Reference Date) (GE Healthcare) using the 'Ready-To-Go™ DNA Labelling Beads' (GE Healthcare). Unincorporated nucleotides were removed by separation on a 'Micro Bio-Spin® 30 Chromatography Column' (Bio-Rad).

The labelling efficiency was estimated with a contamination monitor by comparing the reactivity of the Eppendorf tube containing the labelled probe template with the reactivity of the chromatography column . The labelling was considered to be efficient when the probe template was more reactive than the column. If the efficiency of the probe labelling was not sufficient, the probe was not used for hybridization and a new probe labelling took place.

3.8 Hybridization

Two successive hybridizations were performed on each membrane: a first hybridization was performed using the Ph4a748B probe, the membrane was stripped, and a second hybridization was performed using the T-DNA probe. Hybridization with the probe and washing steps were carried out according to Sambrook *et al*.

The hybridization and washing steps were carried out in a hybridization oven with a rotating bottle rack. For each hybridization, 50 mL of pre-hybridization buffer was prepared (6x SSC, 0.5% SDS, 0.1% dextran sulphate and 100 µg/mL denaturated carrier DNA (single-stranded fish sperm DNA, with an average length of 120 - 3000 nucleotides) and preheated to 65°C. The Nylon membrane was rolled with the DNA side facing inwards and placed in a hybridization tube. Twenty-five mL of pre-hybridization buffer was added to the tube and pre-hybridization of the filters proceeded in the rotation oven at 65°C for 1 to 5 hours. The remaining pre-hybridization buffer was used to prepare the hybridization buffer.

The [α-32P]-labelled probe was denaturated by heating for 5 minutes at 95°C and snap-cooling on ice for at least 5 minutes. Next, the denaturated probe was added to the remaining pre-hybridization buffer. The pre-hybridization buffer in the hybridization tube was replaced by this hybridization buffer. The tube was placed in the hybridization oven at 65°C and rotation of the tube rack was started. The membrane was hybridized overnight.

The hybridization solution was removed and washing of the blots started by adding the first wash solution (6X SSC at 65°C). The hybridization tube was placed in the rotation oven for about 20 minutes. The 6 X SSC solution was removed and hybridization wash buffer 2 (2x SSC and 0.1% SDS at 65°C) was added to the hybridization tube. The tube was placed in the hybridization oven for at least 30 minutes at 65°C. This washing step was performed three times. Next, hybridization wash buffer 2 was replaced by the third hybridization wash buffer (0.1x SSC and 0.1% SDS at 65°C). The tube was rotated in the hybridization oven for 15 minutes at 65°C.

The membrane was removed from the rotating bottle and placed on a sheet of blotting paper. The membrane was monitored and if the background signal was unacceptably high, the membrane was washed between 5 and 10 minutes at 65°C with a high stringency wash solution (0.1 X SSC, 0.1 % SDS) in a plastic box. After the washing was completed the membrane was placed between two sheets of blotting paper to remove excess of moisture. The membrane was not allowed to dry completely as this would hamper stripping of the membrane.

3.9 Autoradiography and stripping of blots

The hybridized fragments were visualized by means of autoradiography. The lower part of an X-ray cassette, containing a phosphorescent alignment sticker was covered with SaranWrap™ . The radioactive hybridized membrane was placed on the sheet of SaranWrap™ and covered with a second sheet of SaranWrap™ avoiding air bubbles between the two sheets. An autoradiography was established by exposing the membrane to a sheet of Kodak BioMax MR film (Sigma-Aldrich), which was at least several inches larger than the membrane. The X-ray cassette was placed in the ultrafreezer or at room-temperature for an appropriate time period (usually overnight till a few days). The film was developed. The image on the film was aligned with the phosphorescent sticker to achieve correct orientation of the X-Ray film. The positions of the gel wells that are marked on the membrane were traced onto the autoradiogram. The film was scanned electronically.

After each hybridization the hybridized membrane was removed from the X-ray cassette, rolled up, put into a cylinder, suffused with a boiling 0.5% SDS solution and cooled down to room temperature. The membrane was stored between Whatman 3MM paper sheets.

4. RESULTS AND DISCUSSION

A schematic presentation of the hybridization strategy is shown in Figure 2. The hybridization results are presented in Figures 3 through 18. A summary of the results obtained is presented in Table 4.

The size of the hybridization fragments was determined using the GeneTools software (Syngene). Conclusions were based on the number of hybridization fragments and the fragment sizes. No conclusions were drawn from the relative intensities of the hybridization signals. The differences in fragment intensity observed in certain samples are the result of slight differences in the amount of genomic DNA loaded on the

gels, and do not impact data interpretation. Slight differences in fragment sizes (e.g. the 947 bp *Hin*dIII fragment of FG72 genomic DNA samples and the 947 bp *Hin*dIII fragment of pSF10 plasmid DNA) are due to technical artifacts and do not impact data interpretation.

The negative control (*Hin*dIII digested genomic DNA prepared from non-transgenic plants of variety JACK) showed no hybridization, confirming the absence of background hybridization.

The positive control (*Hin*dIII digested genomic DNA prepared from a non-transgenic *Glycine max* plant (variety JACK) supplemented with an equimolar amount of *Hin*dIII digested pSF10 plasmid) showed after hybridization with the T-DNA probe the expected hybridization fragments of 3420 bp and 2961 bp. The expected 947 bp fragment of the positive control was sometimes very weak after hybridization with the T-DNA probe, however after hybridization with the Ph4a748B probe, this fragment was visible. This has no impact on the outcome of the results.

The results obtained with the positive control indicate that the Southern blot analysis was performed under conditions that allow for the effective detection of the target sequences.

In all tested *Glycine max* FG72 samples, the presence of the expected 5' and 3' integration fragments, the internal fragments and the presence of the 158 bases of Ph4a748 promoter sequences located next to the translocated sequence was confirmed. This demonstrates the stability of the *Glycine max* transformation event FG72 at the genomic level in different generations, genetic backgrounds and environments.

5. CONCLUSION

To demonstrate the structural stability of transformation event FG72, plants from different generations, different backgrounds and different environments were tested by means of Southern blot analysis. The isolated genomic DNA from the respective individual plants was digested with the restriction enzyme *Hin*dIII, which generates a restriction profile that is unique for FG72.

The obtained Southern blot profile was identical for all samples, which demonstrates the stability of transformation event FG72 at the genomic level in different generations, different environments and different backgrounds.

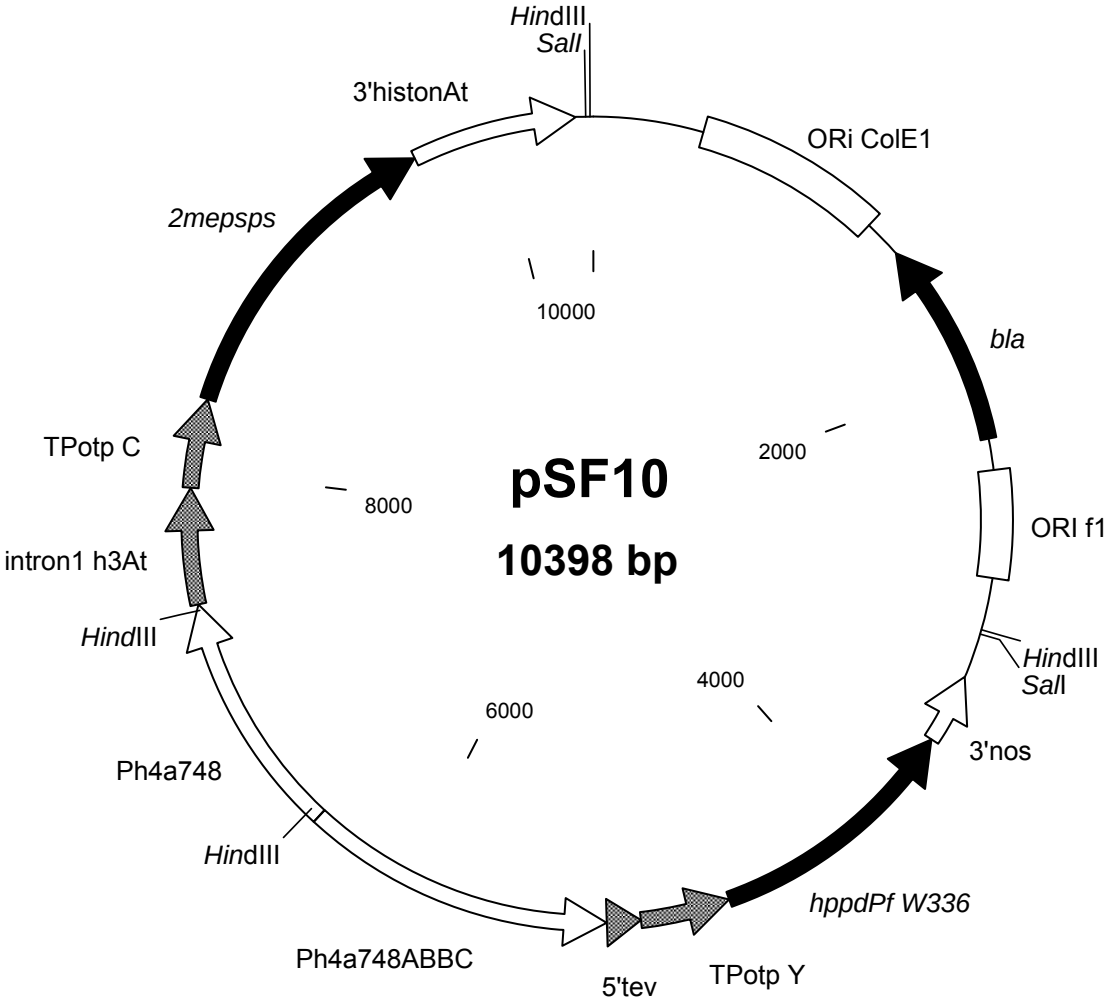

Figure 1: Map of vector pSF10

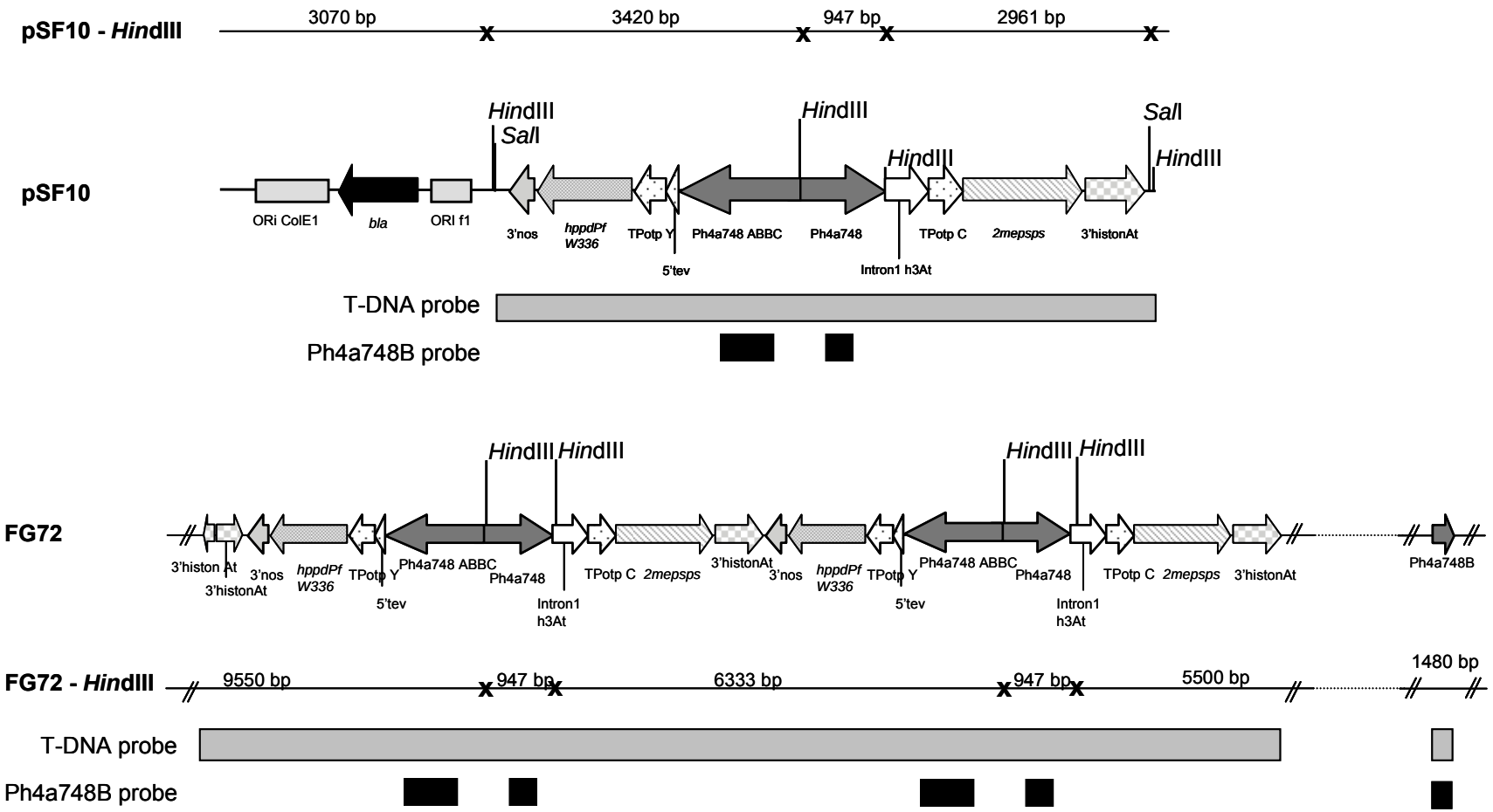

Figure 2: Schematic drawing of hybridization strategy for the demonstration of the structural stability of FG72 transgenic plants

Table 2: Probes used to assess the stability of FG72

Probe template ID	Description	Size probe template (bp)	Position in pSF10 (bp)	Template DNA	Primer pair / Restriction digest	Concentration
204	T-DNA probe	7204	3142 → 10345	pSF10	*Sac*I/*Sma*I	40 ng/µL
206	Ph4a748B	430	6866 → 7295	pSF10	JDB018	160 ng/µL
					JDB019	

Table 3: Information on primers used for Ph4a748B probe template preparation

Primer*	Primer sequence (5' → 3')	Primer position in pSF10 (bp)
JDB018	Critical Confidential Information removed	6866 → 6891 5997← 6022*
JDB019	Critical Confidential Information removed	7269 ← 7295 5260 → 5286*

* Primers JDB018 and JDB019 have 2 possible priming sites in plasmid pSF10. The priming sites indicated with * are not used for the probe template preparation.

Table 4. Southern Blot Analyses – FG72 - Summary of expected and obtained hybridization fragments

Samples	Condition tested	Seed lot	N° of plants	Expected T-DNA or plasmid fragment sizes	Fragment description	Ph4a748B probe		T-DNA probe	
						Exp.	Obt.	Exp.	Obt
FG72 - *Hin*dIII	Location Adel, Iowa	HT08SOY002-05-32	22						
	Location Osborn, Missouri	HT08SOY002-07-32	22						
	Location Fithian, Illinois	HT08SOY002-08-32	21	9550 bp [d]	5' integration fr.	Yes	Yes	Yes	Yes
	Location Sharpsville, Indiana	HT08SOY002-09-32	16	947 bp	internal fragment	Yes	Yes	Yes	Yes
	Background 3068115-48 x Jack	7BD60008	21	6333 bp	internal fragment	Yes	Yes	Yes	Yes
	Background 3066617-48 x Jack	7BD60018	22	5500 bp [d]	3' integration fr.	No	No	Yes	Yes
	Generation T2	FG72a-T2, FG72b-T2, FG72c-T2, FG72d,T2	22	1480 bp [d]	3' junction translocation	Yes	Yes [a]	Yes [b]	No [c]
	Generation T7	FG72-x-x-14-5-1-6-x T7	13						
Non transgenic Jack - *Hin*dIII	Non-transgenic variety Jack			/	Negative control	/	/	/	/
Non transgenic Jack - *Hin*dIII + pSF10 - *Hin*dIII	Non-transgenic variety Jack + equimolar amount pSF10			3420 bp	positive control	Yes	Yes	Yes	Yes
				947 bp	positive control	Yes	Yes	Yes	No [c]
				2961 bp	positive control	No	No	Yes	Yes
				3070 bp	positive control	No	No	No	No

[a] In some hybridizations this fragment is very weak, but present for all samples

[b] The overlap between the probe and the fragment can be too small to visualize this fragment

[c] Not always visible after hybridization with T-DNA probe but presence is confirmed after hybridization with probe Ph4a748B.

[d] Expected fragment sizes as determined in the detailed insert characterization study (Verhaeghe S.; 2010).

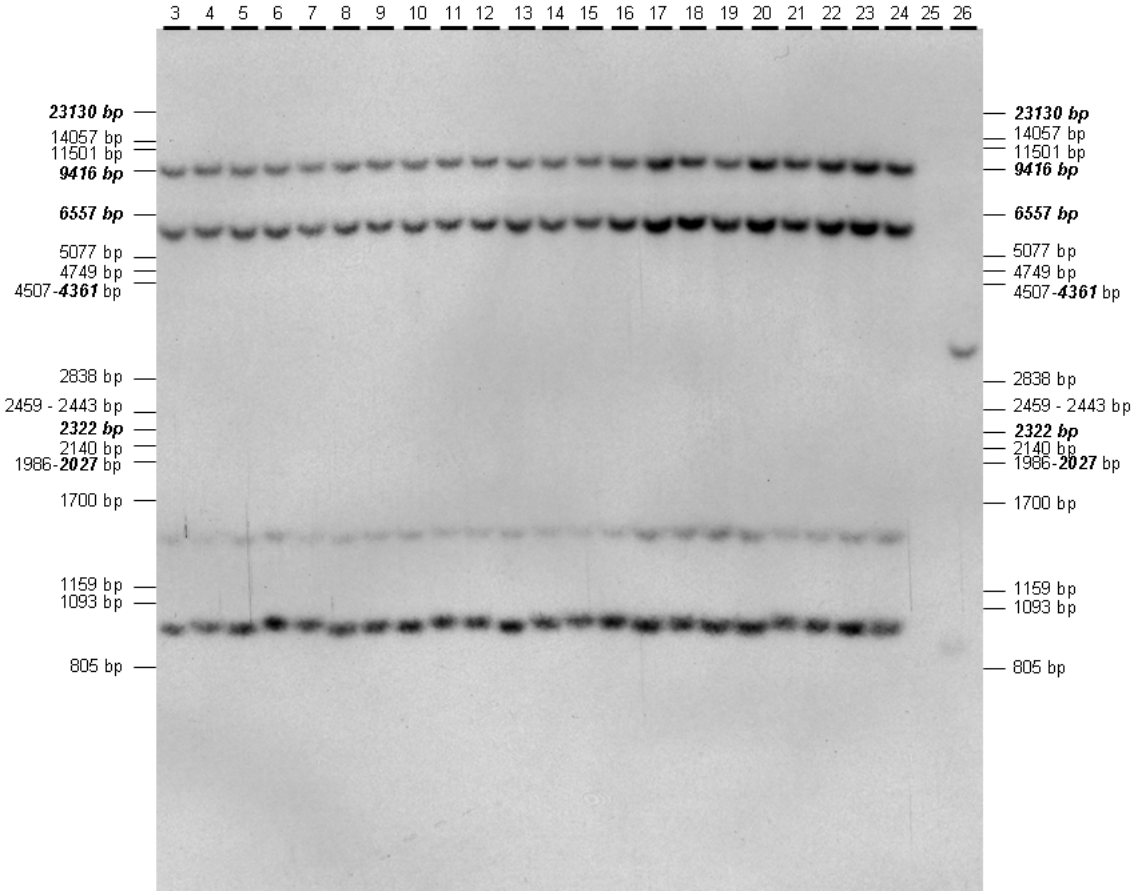

Figure 3: Demonstration of the stability of FG72 – seed lot HT08SOY002-05-32 (Environments: Adel, Probe: Ph4a748B)

Gel: NS-TV/BA00629/05
Probe: Ph4a748B
Film: HYB-TV/BA00629/05/01-F3

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-05-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 143 - *Hin*dIII
Lane 4: FG72 plant 145 - *Hin*dIII
Lane 5: FG72 plant 146 - *Hin*dIII
Lane 6: FG72 plant 147 - *Hin*dIII
Lane 7: FG72 plant 148 - *Hin*dIII
Lane 8: FG72 plant 149 - *Hin*dIII
Lane 9: FG72 plant 150 - *Hin*dIII
Lane 10: FG72 plant 151 - *Hin*dIII
Lane 11: FG72 plant 152 - *Hin*dIII
Lane 12: FG72 plant 153 - *Hin*dIII
Lane 13: FG72 plant 154 - *Hin*dIII
Lane 14: FG72 plant 155 - *Hin*dIII
Lane 15: FG72 plant 156 - *Hin*dIII
Lane 16: FG72 plant 158 - *Hin*dIII
Lane 17: FG72 plant 159 - *Hin*dIII
Lane 18: FG72 plant 160 - *Hin*dIII
Lane 19: FG72 plant 161 - *Hin*dIII
Lane 20: FG72 plant 162 - *Hin*dIII
Lane 21: FG72 plant 163 - *Hin*dIII
Lane 22: FG72 plant 164 - *Hin*dIII
Lane 23: FG72 plant 165 - *Hin*dIII
Lane 24: FG72 plant 169 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

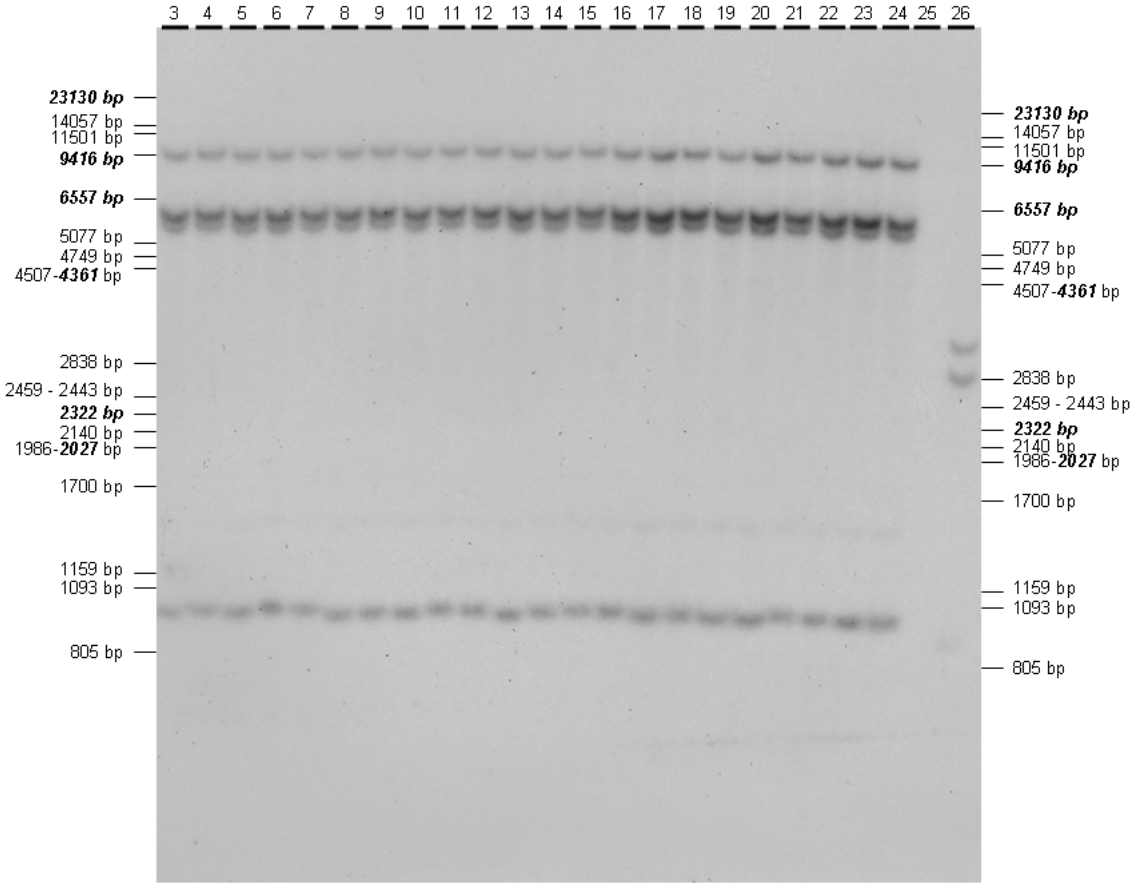

Figure 4: Demonstration of the stability of FG72 – seed lot HT08SOY002-05-32 (Environments: Adel, Probe: T-DNA)

Gel: NS-TV/BA00629/05
Probe: T-DNA
Film: HYB-TV/BA00629/05/03-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-05-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 143 - *Hin*dIII
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Lane 8: FG72 plant 149 - *Hin*dIII
Lane 9: FG72 plant 150 - *Hin*dIII
Lane 10: FG72 plant 151 - *Hin*dIII
Lane 11: FG72 plant 152 - *Hin*dIII
Lane 12: FG72 plant 153 - *Hin*dIII
Lane 13: FG72 plant 154 - *Hin*dIII
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Lane 17: FG72 plant 159 - *Hin*dIII
Lane 18: FG72 plant 160 - *Hin*dIII
Lane 19: FG72 plant 161 - *Hin*dIII
Lane 20: FG72 plant 162 - *Hin*dIII
Lane 21: FG72 plant 163 - *Hin*dIII
Lane 22: FG72 plant 164 - *Hin*dIII
Lane 23: FG72 plant 165 - *Hin*dIII
Lane 24: FG72 plant 169 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 – *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

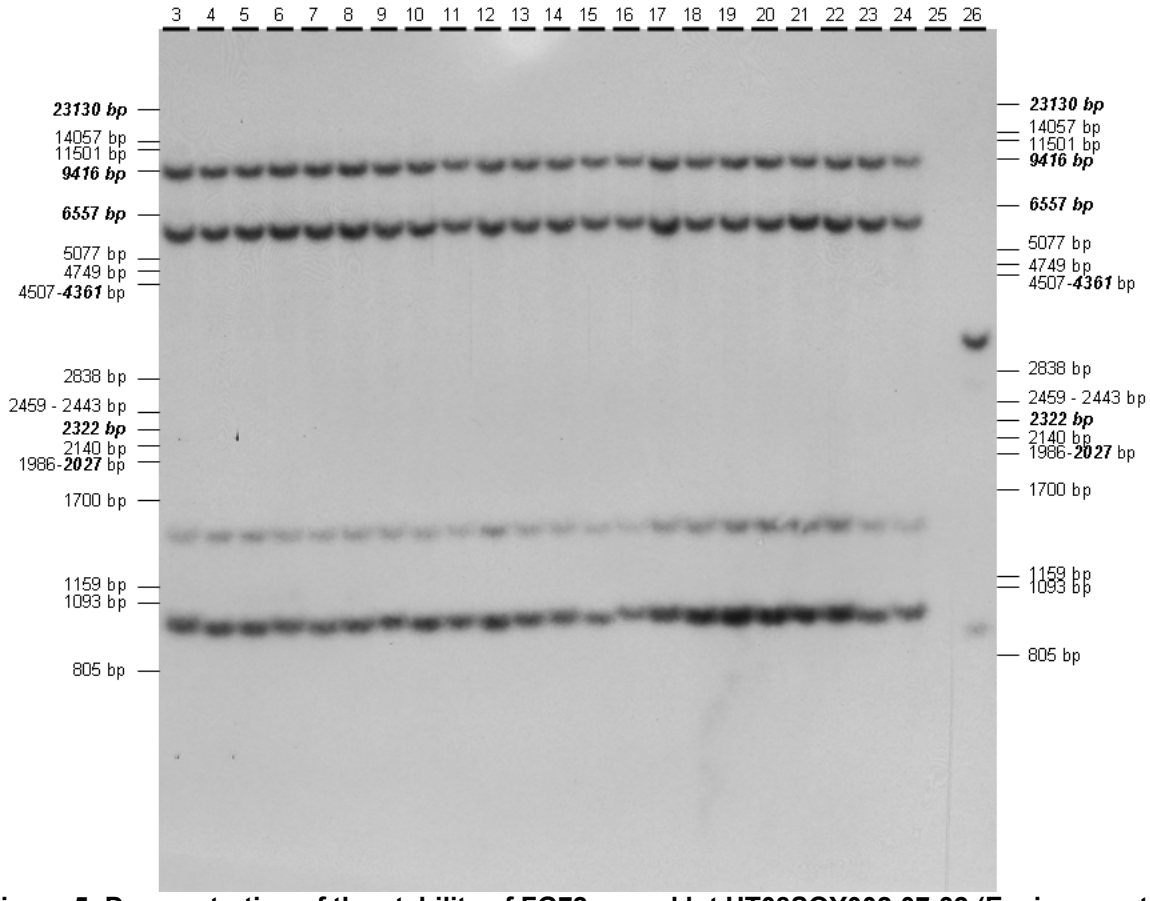

Figure 5: Demonstration of the stability of FG72 – seed lot HT08SOY002-07-32 (Environments: Osborn, Probe: Ph4a748B)

Gel: NS-TV/BA00629/06
Probe: Ph4a748B
Film: HYB-TV/BA00629/06/03-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-07-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 176 - *Hin*dIII
Lane 4: FG72 plant 177 - *Hin*dIII
Lane 5: FG72 plant 178 - *Hin*dIII
Lane 6: FG72 plant 179 - *Hin*dIII
Lane 7: FG72 plant 180 - *Hin*dIII
Lane 8: FG72 plant 181 - *Hin*dIII
Lane 9: FG72 plant 182 - *Hin*dIII
Lane 10: FG72 plant 183 - *Hin*dIII
Lane 11: FG72 plant 185 - *Hin*dIII
Lane 12: FG72 plant 202 - *Hin*dIII
Lane 13: FG72 plant 203 - *Hin*dIII
Lane 14: FG72 plant 184 - *Hin*dIII
Lane 15: FG72 plant 186 - *Hin*dIII
Lane 16: FG72 plant 187 - *Hin*dIII
Lane 17: FG72 plant 188 - *Hin*dIII
Lane 18: FG72 plant 189 - *Hin*dIII
Lane 19: FG72 plant 190 - *Hin*dIII
Lane 20: FG72 plant 197 - *Hin*dIII
Lane 21: FG72 plant 198 - *Hin*dIII
Lane 22: FG72 plant 199 - *Hin*dIII
Lane 23: FG72 plant 200 - *Hin*dIII
Lane 24: FG72 plant 201 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

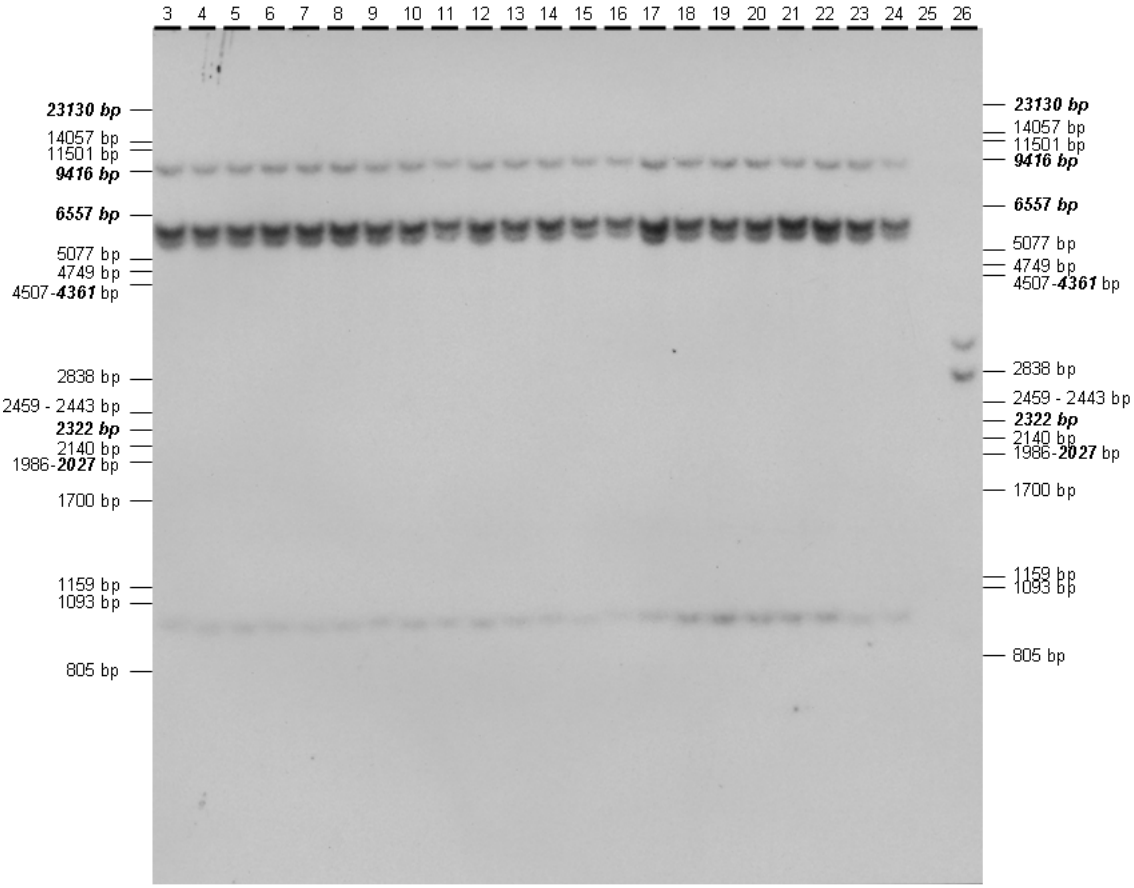

Figure 6: Demonstration of the stability of FG72 – seed lot HT08SOY002-07-32 (Environments: Osborn, Probe: T-DNA)

Gel: NS-TV/BA00629/06
Probe: T-DNA
Film: HYB-TV/BA00629/06/02-F4

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-07-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 176 - *Hin*dIII
Lane 4: FG72 plant 177 - *Hin*dIII
Lane 5: FG72 plant 178 - *Hin*dIII
Lane 6: FG72 plant 179 - *Hin*dIII
Lane 7: FG72 plant 180 - *Hin*dIII
Lane 8: FG72 plant 181 - *Hin*dIII
Lane 9: FG72 plant 182 - *Hin*dIII
Lane 10: FG72 plant 183 - *Hin*dIII
Lane 11: FG72 plant 185 - *Hin*dIII
Lane 12: FG72 plant 202 - *Hin*dIII
Lane 13: FG72 plant 203 - *Hin*dIII
Lane 14: FG72 plant 184 - *Hin*dIII
Lane 15: FG72 plant 186 - *Hin*dIII
Lane 16: FG72 plant 187 - *Hin*dIII
Lane 17: FG72 plant 188 - *Hin*dIII
Lane 18: FG72 plant 189 - *Hin*dIII
Lane 19: FG72 plant 190 - *Hin*dIII
Lane 20: FG72 plant 197 - *Hin*dIII
Lane 21: FG72 plant 198 - *Hin*dIII
Lane 22: FG72 plant 199 - *Hin*dIII
Lane 23: FG72 plant 200 - *Hin*dIII
Lane 24: FG72 plant 201 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

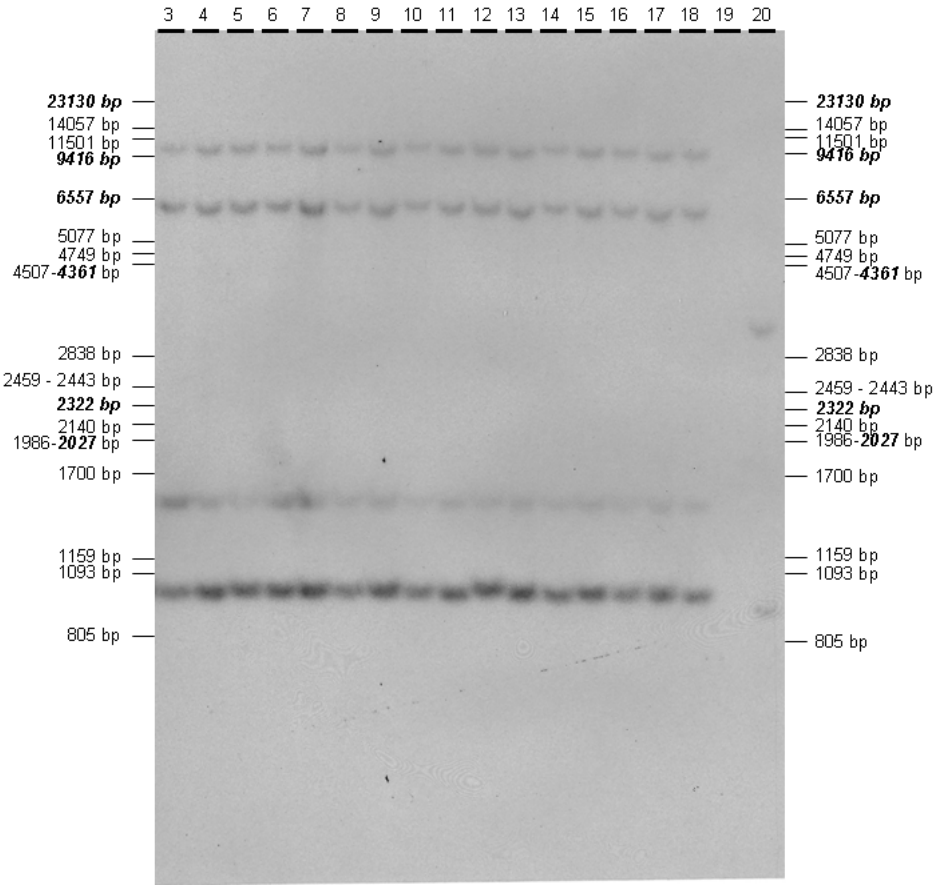

Figure 7: Demonstration of the stability of FG72 – seed lot HT08SOY002-09-32 (Environments: Sharpsville, Probe: Ph4a748B)

Gel: NS-TV/BA00629/08
Probe: Ph4a748B
Film: HYB-TV/BA00629/08/01-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-09-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 84 - *Hin*dIII
Lane 4: FG72 plant 87 - *Hin*dIII
Lane 5: FG72 plant 88 - *Hin*dIII
Lane 6: FG72 plant 90 - *Hin*dIII
Lane 7: FG72 plant 91 - *Hin*dIII
Lane 8: FG72 plant 92 - *Hin*dIII
Lane 9: FG72 plant 93 - *Hin*dIII
Lane 10: FG72 plant 94 - *Hin*dIII
Lane 11: FG72 plant 95 - *Hin*dIII
Lane 12: FG72 plant 96 - *Hin*dIII
Lane 13: FG72 plant 97 - *Hin*dIII
Lane 14: FG72 plant 98 - *Hin*dIII
Lane 15: FG72 plant 99 - *Hin*dIII
Lane 16: FG72 plant 100 - *Hin*dIII
Lane 17: FG72 plant 101 - *Hin*dIII
Lane 18: FG72 plant 102 - *Hin*dIII
Lane 19: Non-transgenic variety JACK - *Hin*dIII
Lane 20: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 21: Phage Lambda – *Pst*I digested
Lane 22: Phage Lambda – *Hin*dIII digested

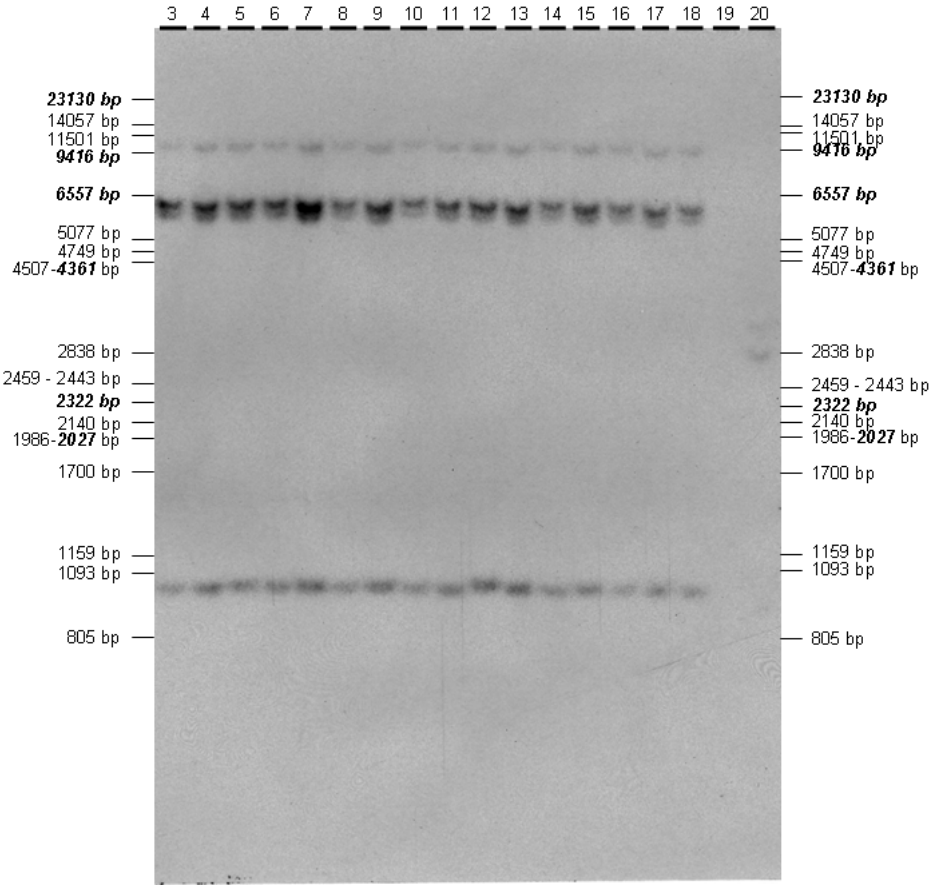

Figure 8: Demonstration of the stability of FG72 – seed lot HT08SOY002-09-32 (Environments: Sharpsville, Probe: T-DNA)

Gel: NS-TV/BA00629/08
Probe: T-DNA
Film: HYB-TV/BA00629/08/02-F4

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-09-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 84 - *Hin*dIII
Lane 4: FG72 plant 87 - *Hin*dIII
Lane 5: FG72 plant 88 - *Hin*dIII
Lane 6: FG72 plant 90 - *Hin*dIII
Lane 7: FG72 plant 91 - *Hin*dIII
Lane 8: FG72 plant 92 - *Hin*dIII
Lane 9: FG72 plant 93 - *Hin*dIII
Lane 10: FG72 plant 94 - *Hin*dIII
Lane 11: FG72 plant 95 - *Hin*dIII
Lane 12: FG72 plant 96 - *Hin*dIII
Lane 13: FG72 plant 97 - *Hin*dIII
Lane 14: FG72 plant 98 - *Hin*dIII
Lane 15: FG72 plant 99 - *Hin*dIII
Lane 16: FG72 plant 100 - *Hin*dIII
Lane 17: FG72 plant 101 - *Hin*dIII
Lane 18: FG72 plant 102 - *Hin*dIII
Lane 19: Non-transgenic variety JACK - *Hin*dIII
Lane 20: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 21: Phage Lambda – *Pst*I digested
Lane 22: Phage Lambda – *Hin*dIII digested

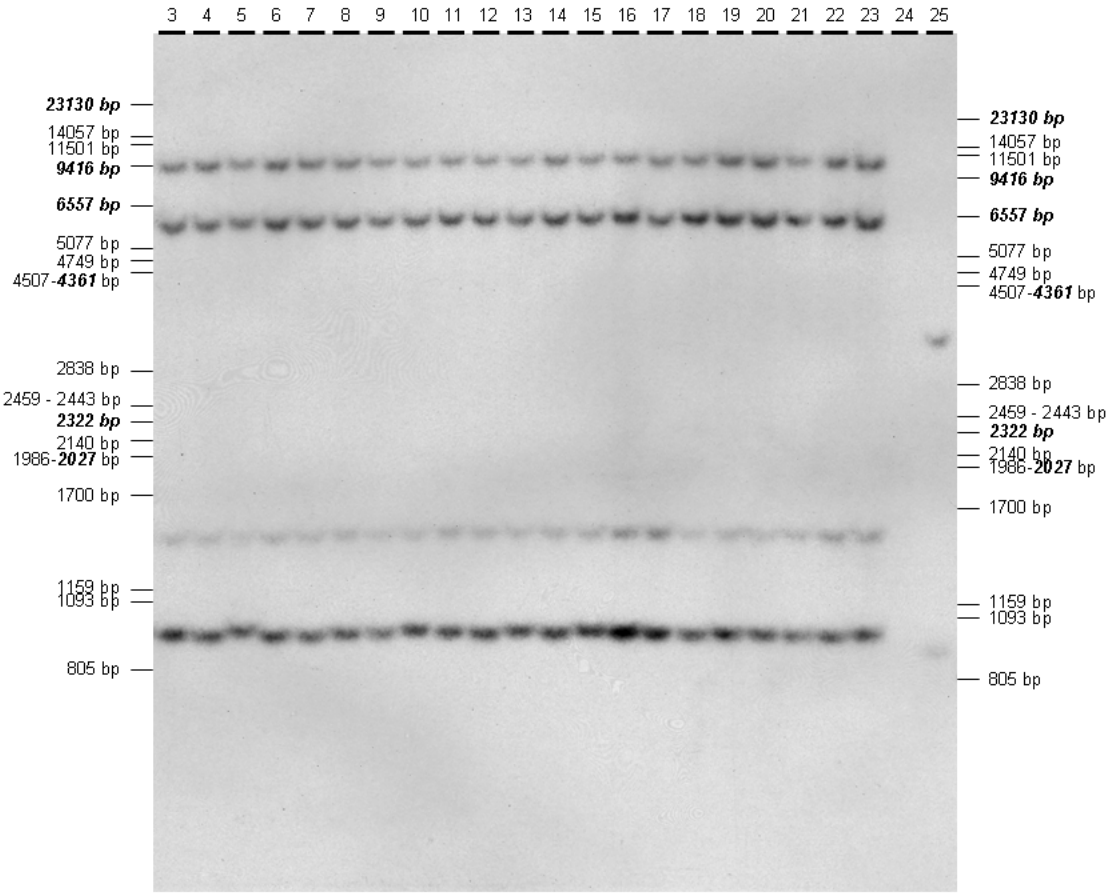

Figure 9: Demonstration of the stability of FG72 – seed lot HT08SOY002-08-32 (Environments: Fithian, Probe: Ph4a748B)

Gel: NS-TV/BA00629/07
Probe: Ph4a748B
Film: HYB-TV/BA00629/07/04-F1

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-08-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hind*III and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 108 - *Hind*III
Lane 4: FG72 plant 109 - *Hind*III
Lane 5: FG72 plant 110 - *Hind*III
Lane 6: FG72 plant 111 - *Hind*III
Lane 7: FG72 plant 112 - *Hind*III
Lane 8: FG72 plant 113 - *Hind*III
Lane 9: FG72 plant 115 - *Hind*III
Lane 10: FG72 plant 116 - *Hind*III
Lane 11: FG72 plant 117 - *Hind*III
Lane 12: FG72 plant 119 - *Hind*III
Lane 13: FG72 plant 120 - *Hind*III
Lane 14: FG72 plant 121 - *Hind*III
Lane 15: FG72 plant 122 - *Hind*III
Lane 16: FG72 plant 123 - *Hind*III
Lane 17: FG72 plant 124 - *Hind*III
Lane 18: FG72 plant 125 - *Hind*III
Lane 19: FG72 plant 127 - *Hind*III
Lane 20: FG72 plant 128 - *Hind*III
Lane 21: FG72 plant 129 - *Hind*III
Lane 22: FG72 plant 130 - *Hind*III
Lane 23: FG72 plant 131 - *Hind*III
Lane 24: Non-transgenic variety JACK - *Hind*III
Lane 25: Non-transgenic variety JACK - *Hind*III + equimolar amount pSF10 - *Hind*III
Lane 26: Phage Lambda – *Pst*I digested
Lane 27: Phage Lambda – *Hind*III digested

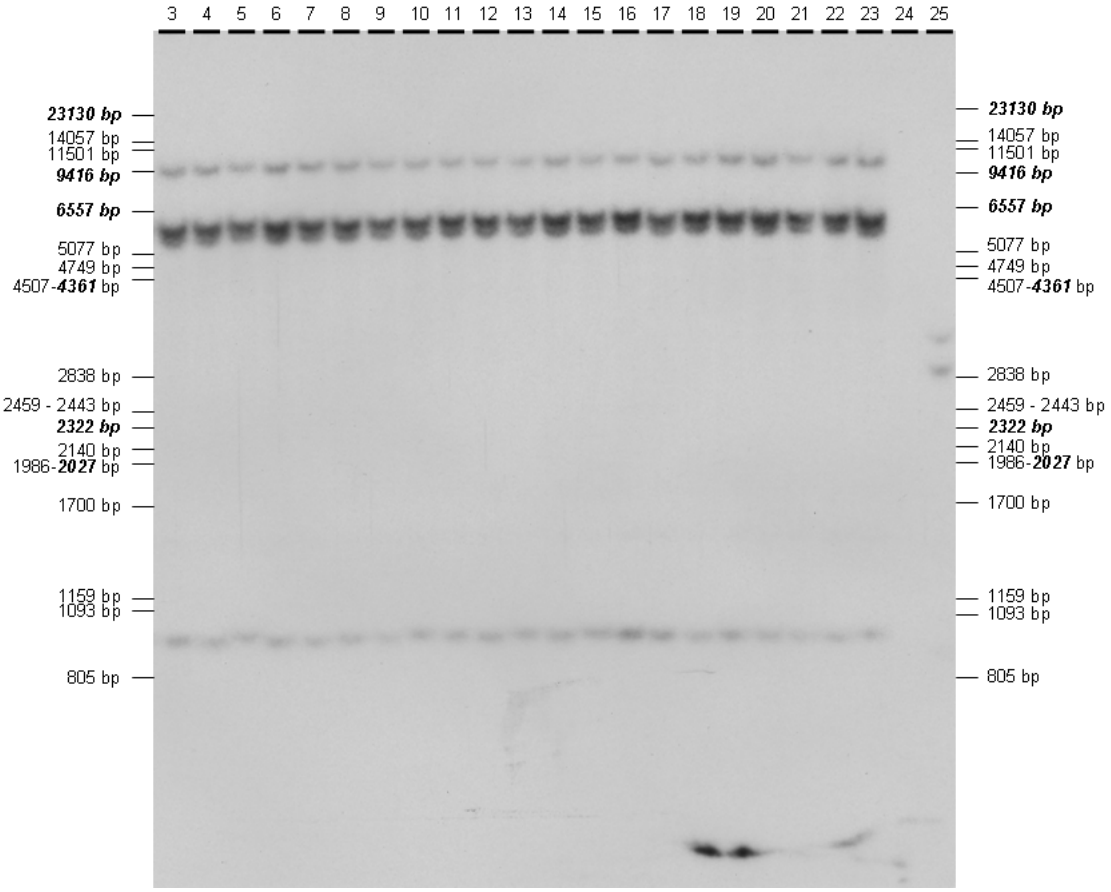

Figure 10: Demonstration of the stability of FG72 – seed lot HT08SOY002-08-32 (Environments: Fithian, Probe: T-DNA)

Gel: NS-TV/BA00629/07
Probe: T-DNA
Film: HYB-TV/BA00629/07/05-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot HT08SOY002-08-32) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 108 - *Hin*dIII
Lane 4: FG72 plant 109 - *Hin*dIII
Lane 5: FG72 plant 110 - *Hin*dIII
Lane 6: FG72 plant 111 - *Hin*dIII
Lane 7: FG72 plant 112 - *Hin*dIII
Lane 8: FG72 plant 113 - *Hin*dIII
Lane 9: FG72 plant 115 - *Hin*dIII
Lane 10: FG72 plant 116 - *Hin*dIII
Lane 11: FG72 plant 117 - *Hin*dIII
Lane 12: FG72 plant 119 - *Hin*dIII
Lane 13: FG72 plant 120 - *Hin*dIII
Lane 14: FG72 plant 121 - *Hin*dIII
Lane 15: FG72 plant 122 - *Hin*dIII
Lane 16: FG72 plant 123 - *Hin*dIII
Lane 17: FG72 plant 124 - *Hin*dIII
Lane 18: FG72 plant 125 - *Hin*dIII
Lane 19: FG72 plant 127 - *Hin*dIII
Lane 20: FG72 plant 128 - *Hin*dIII
Lane 21: FG72 plant 129 - *Hin*dIII
Lane 22: FG72 plant 130 - *Hin*dIII
Lane 23: FG72 plant 131 - *Hin*dIII
Lane 24: Non-transgenic variety JACK - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 26: Phage Lambda – *Pst*I digested
Lane 27: Phage Lambda – *Hin*dIII digested

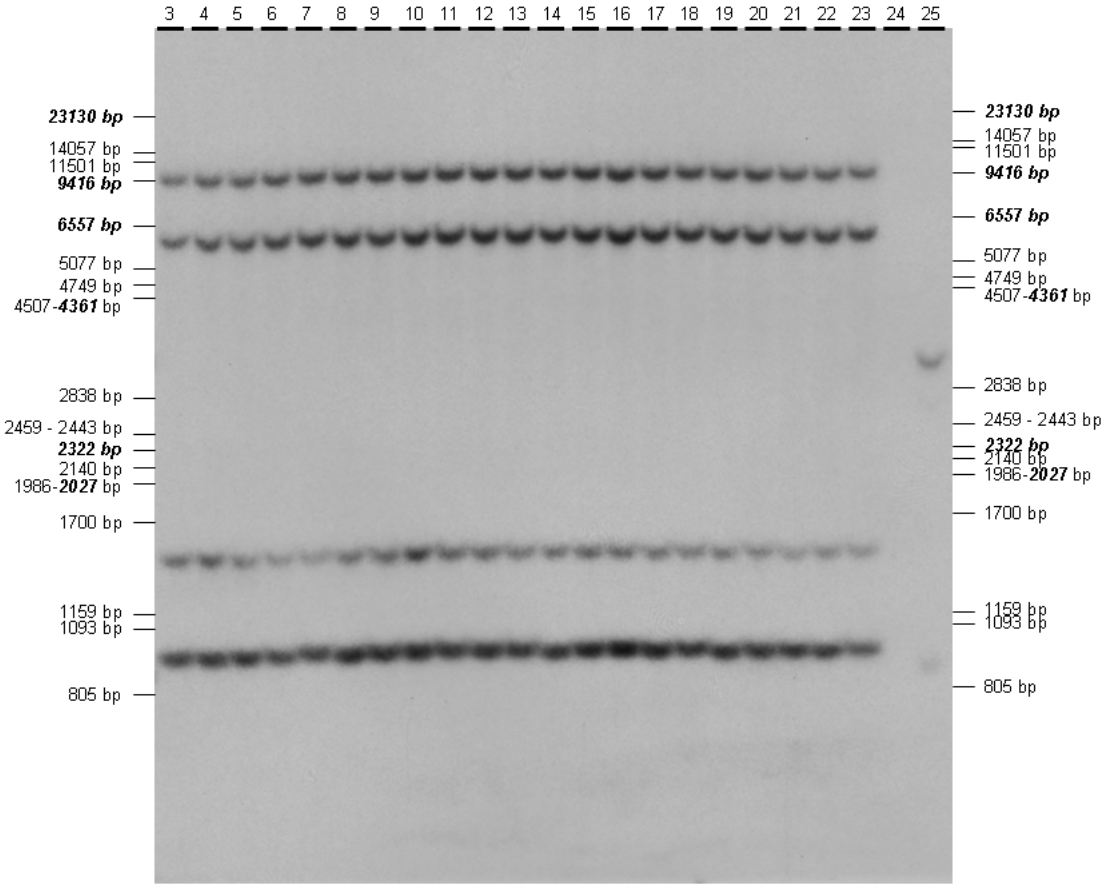

Figure 11: Demonstration of the stability of FG72 – seed lot 7BD60018 (Backgrounds: 3068115-48 X Jack, Probe: Ph4a748B)

Gel: NS-TV/BA00628/02
Probe: Ph4a748B
Film: HYB-TV/BA00628/02/01-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot 7BD60018) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 41 - *Hin*dIII
Lane 4: FG72 plant 56 - *Hin*dIII
Lane 5: FG72 plant 61 - *Hin*dIII
Lane 6: FG72 plant 38 - *Hin*dIII
Lane 7: FG72 plant 39 - *Hin*dIII
Lane 8: FG72 plant 40 - *Hin*dIII
Lane 9: FG72 plant 42 - *Hin*dIII
Lane 10: FG72 plant 43 - *Hin*dIII
Lane 11: FG72 plant 45 - *Hin*dIII
Lane 12: FG72 plant 46 - *Hin*dIII
Lane 13: FG72 plant 47 - *Hin*dIII
Lane 14: FG72 plant 48 - *Hin*dIII
Lane 15: FG72 plant 49 - *Hin*dIII
Lane 16: FG72 plant 50 - *Hin*dIII
Lane 17: FG72 plant 51 - *Hin*dIII
Lane 18: FG72 plant 52 - *Hin*dIII
Lane 19: FG72 plant 55 - *Hin*dIII
Lane 20: FG72 plant 57 - *Hin*dIII
Lane 21: FG72 plant 58 - *Hin*dIII
Lane 22: FG72 plant 59 - *Hin*dIII
Lane 23: FG72 plant 60 - *Hin*dIII
Lane 24: Non-transgenic variety JACK - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 26: Phage Lambda – *Pst*I digested
Lane 27: Phage Lambda – *Hin*dIII digested

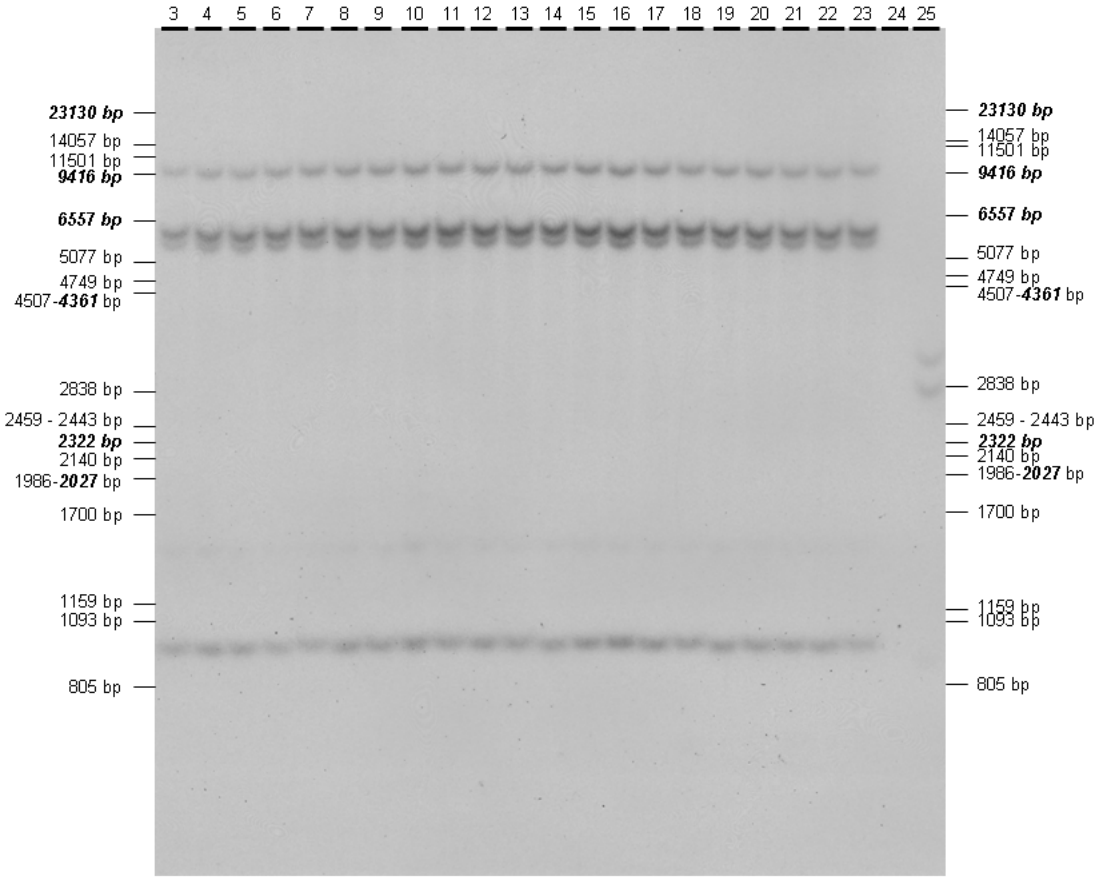

Figure 12: Demonstration of the stability of FG72 – seed lot 7BD60018 (Backgrounds: 3068115-48 X Jack, Probe: T-DNA)

Gel: NS-TV/BA00628/02
Probe: T-DNA
Film: HYB-TV/BA00628/02/02-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot 7BD60018) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 41 - *Hin*dIII
Lane 4: FG72 plant 56 - *Hin*dIII
Lane 5: FG72 plant 61 - *Hin*dIII
Lane 6: FG72 plant 38 - *Hin*dIII
Lane 7: FG72 plant 39 - *Hin*dIII
Lane 8: FG72 plant 40 - *Hin*dIII
Lane 9: FG72 plant 42 - *Hin*dIII
Lane 10: FG72 plant 43 - *Hin*dIII
Lane 11: FG72 plant 45 - *Hin*dIII
Lane 12: FG72 plant 46 - *Hin*dIII
Lane 13: FG72 plant 47 - *Hin*dIII
Lane 14: FG72 plant 48 - *Hin*dIII
Lane 15: FG72 plant 49 - *Hin*dIII
Lane 16: FG72 plant 50 - *Hin*dIII
Lane 17: FG72 plant 51 - *Hin*dIII
Lane 18: FG72 plant 52 - *Hin*dIII
Lane 19: FG72 plant 55 - *Hin*dIII
Lane 20: FG72 plant 57 - *Hin*dIII
Lane 21: FG72 plant 58 - *Hin*dIII
Lane 22: FG72 plant 59 - *Hin*dIII
Lane 23: FG72 plant 60 - *Hin*dIII
Lane 24: Non-transgenic variety JACK - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 26: Phage Lambda – *Pst*I digested
Lane 27: Phage Lambda – *Hin*dIII digested

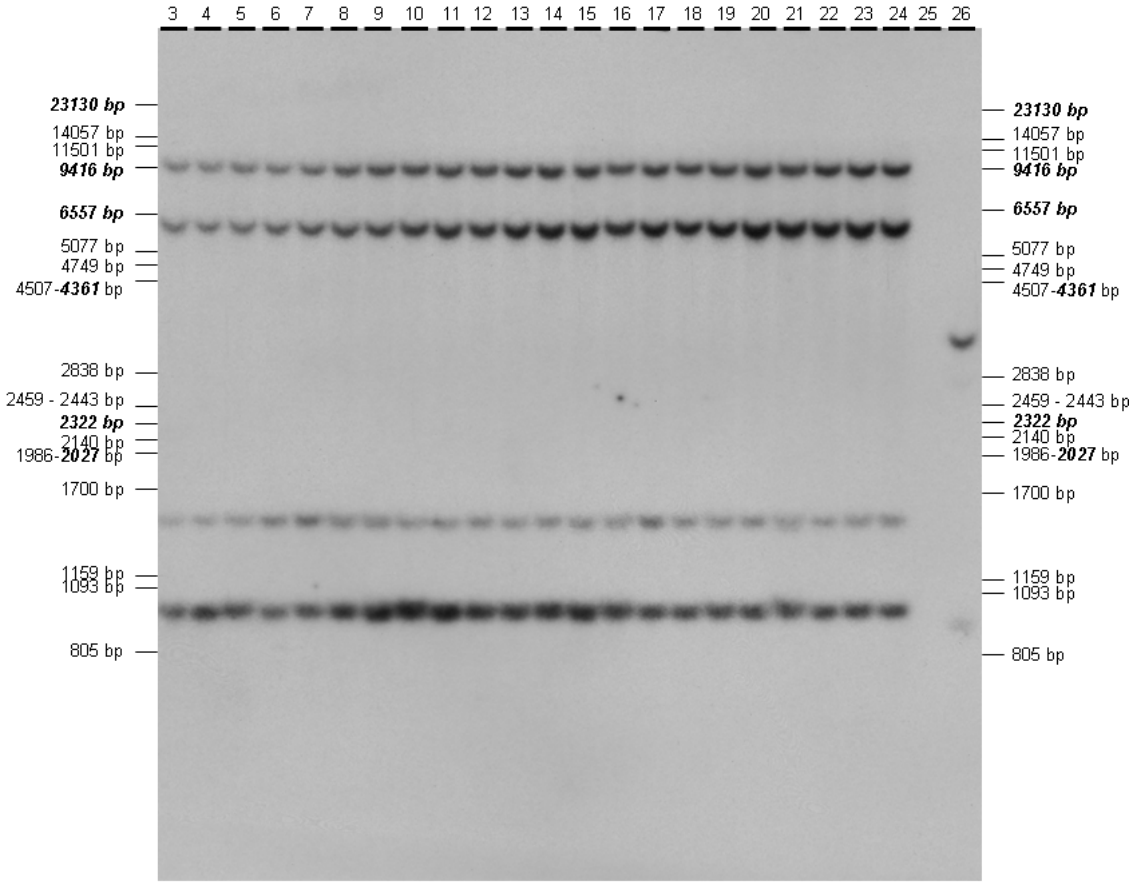

Figure 13: Demonstration of the stability of FG72 – seed lot 7BD60008 (Backgrounds: 3066617-48 X Jack, Probe: Ph4a748B)

Gel: NS-TV/BA00628/01
Probe: Ph4a748B
Film: HYB-TV/BA00628/01/01-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot 7BD60008) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 6 - *Hin*dIII
Lane 4: FG72 plant 16 - *Hin*dIII
Lane 5: FG72 plant 1 - *Hin*dIII
Lane 6: FG72 plant 2 - *Hin*dIII
Lane 7: FG72 plant 3 - *Hin*dIII
Lane 8: FG72 plant 4 - *Hin*dIII
Lane 9: FG72 plant 5 - *Hin*dIII
Lane 10: FG72 plant 7 - *Hin*dIII
Lane 11: FG72 plant 8 - *Hin*dIII
Lane 12: FG72 plant 9 - *Hin*dIII
Lane 13: FG72 plant 10 - *Hin*dIII
Lane 14: FG72 plant 12 - *Hin*dIII
Lane 15: FG72 plant 13 - *Hin*dIII
Lane 16: FG72 plant 14 - *Hin*dIII
Lane 17: FG72 plant 15 - *Hin*dIII
Lane 18: FG72 plant 17 - *Hin*dIII
Lane 19: FG72 plant 18 - *Hin*dIII
Lane 20: FG72 plant 19 - *Hin*dIII
Lane 21: FG72 plant 20 - *Hin*dIII
Lane 22: FG72 plant 21 - *Hin*dIII
Lane 23: FG72 plant 22 - *Hin*dIII
Lane 24: FG72 plant 24 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

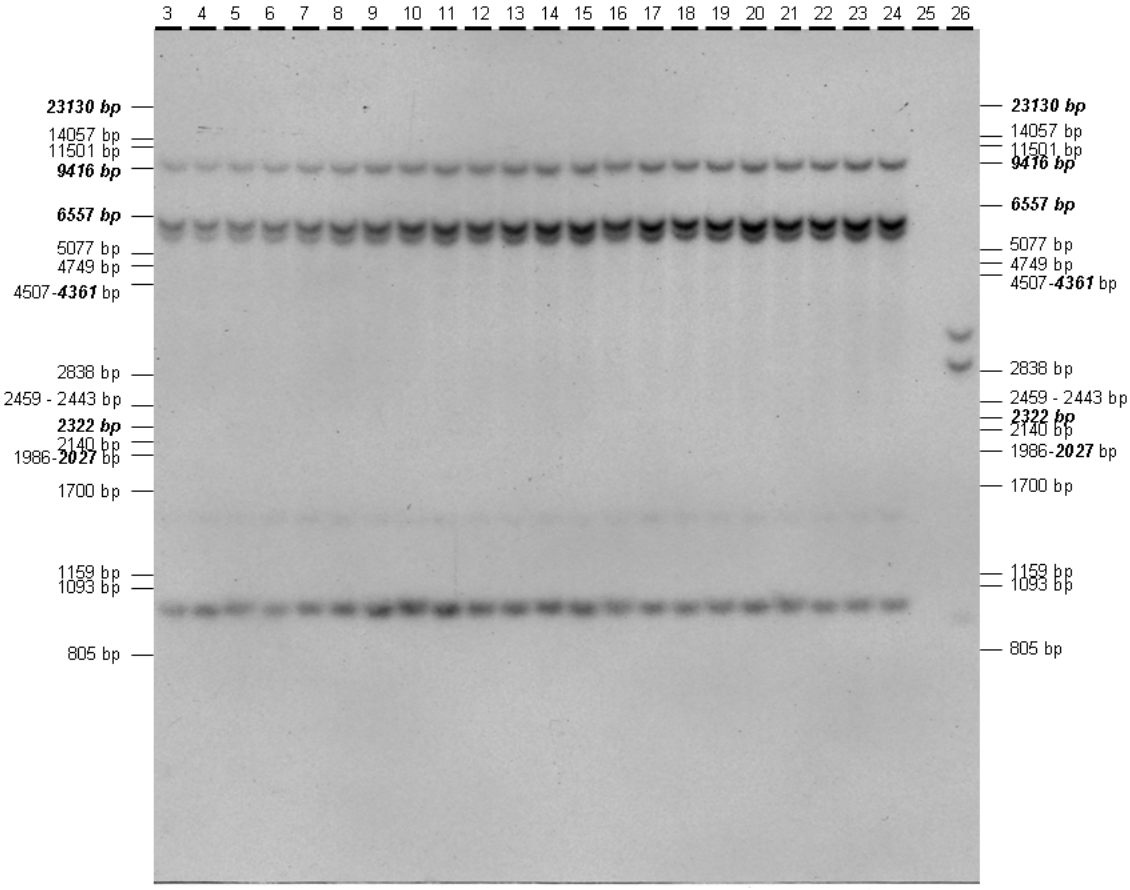

Figure 14: Demonstration of the stability of FG72 – seed lot 7BD60008 (Backgrounds: 3066617-48 X Jack, Probe: T-DNA)

Gel: NS-TV/BA00628/01
Probe: T-DNA
Film: HYB-TV/BA00628/01/02-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot 7BD60008) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 6 - *Hin*dIII
Lane 4: FG72 plant 16 - *Hin*dIII
Lane 5: FG72 plant 1 - *Hin*dIII
Lane 6: FG72 plant 2 - *Hin*dIII
Lane 7: FG72 plant 3 - *Hin*dIII
Lane 8: FG72 plant 4 - *Hin*dIII
Lane 9: FG72 plant 5 - *Hin*dIII
Lane 10: FG72 plant 7 - *Hin*dIII
Lane 11: FG72 plant 8 - *Hin*dIII
Lane 12: FG72 plant 9 - *Hin*dIII
Lane 13: FG72 plant 10 - *Hin*dIII
Lane 14: FG72 plant 12 - *Hin*dIII
Lane 15: FG72 plant 13 - *Hin*dIII
Lane 16: FG72 plant 14 - *Hin*dIII
Lane 17: FG72 plant 15 - *Hin*dIII
Lane 18: FG72 plant 17 - *Hin*dIII
Lane 19: FG72 plant 18 - *Hin*dIII
Lane 20: FG72 plant 19 - *Hin*dIII
Lane 21: FG72 plant 20 - *Hin*dIII
Lane 22: FG72 plant 21 - *Hin*dIII
Lane 23: FG72 plant 22 - *Hin*dIII
Lane 24: FG72 plant 24 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

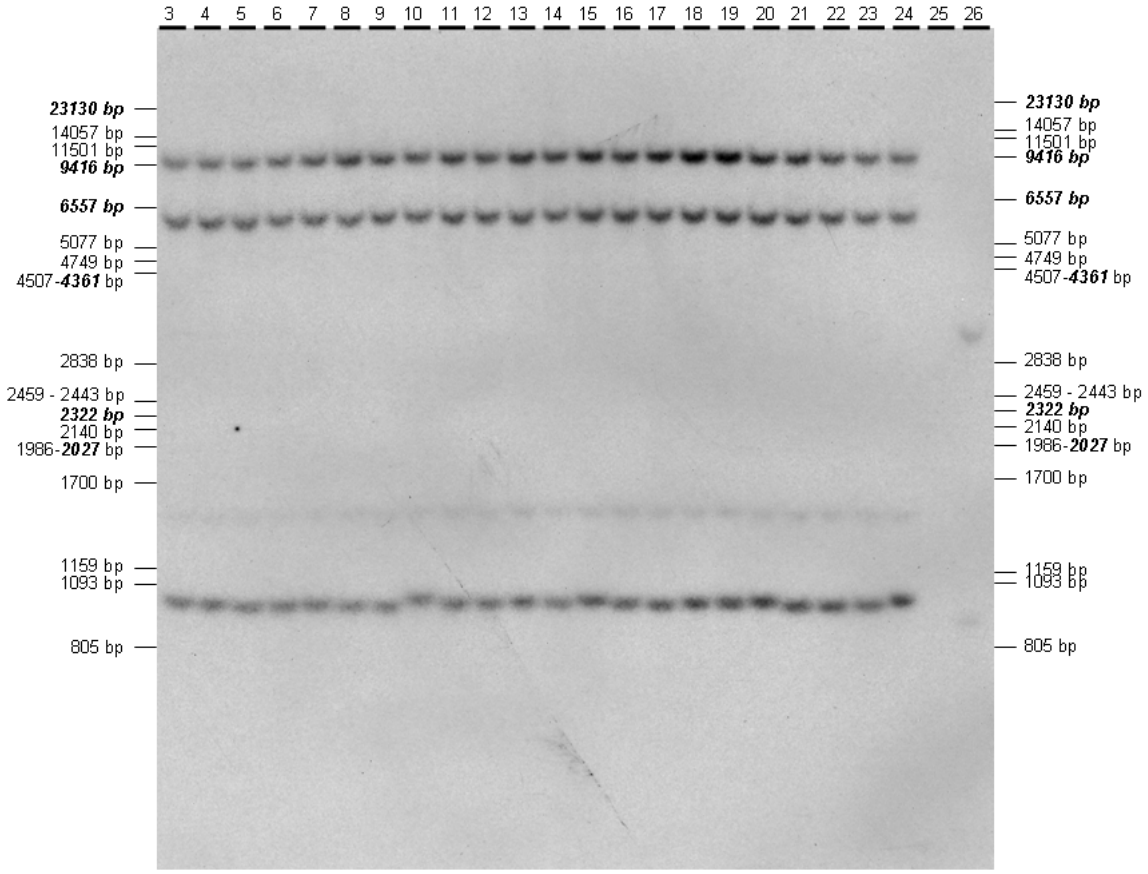

Figure 15: Demonstration of the stability of FG72 – seed lot FG72a-T2 till FG72d-T2 (Generations: T2, Probe: Ph4a748B)

Gel: NS-TV/BA00627/01
Probe: Ph4a748B
Film: HYB-TV/BA00627/01/01-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot FG72a-T2 till FG72d-T2) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 239 - *Hin*dIII
Lane 4: FG72 plant 251 - *Hin*dIII
Lane 5: FG72 plant 273 - *Hin*dIII
Lane 6: FG72 plant 274 - *Hin*dIII
Lane 7: FG72 plant 275 - *Hin*dIII
Lane 8: FG72 plant 276 - *Hin*dIII
Lane 9: FG72 plant 277 - *Hin*dIII
Lane 10: FG72 plant 278 - *Hin*dIII
Lane 11: FG72 plant 279 - *Hin*dIII
Lane 12: FG72 plant 280 - *Hin*dIII
Lane 13: FG72 plant 281 - *Hin*dIII
Lane 14: FG72 plant 282 - *Hin*dIII
Lane 15: FG72 plant 283 - *Hin*dIII
Lane 16: FG72 plant 284 - *Hin*dIII
Lane 17: FG72 plant 285 - *Hin*dIII
Lane 18: FG72 plant 286 - *Hin*dIII
Lane 19: FG72 plant 287 - *Hin*dIII
Lane 20: FG72 plant 288 - *Hin*dIII
Lane 21: FG72 plant 289 - *Hin*dIII
Lane 22: FG72 plant 290 - *Hin*dIII
Lane 23: FG72 plant 291 - *Hin*dIII
Lane 24: FG72 plant 301 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

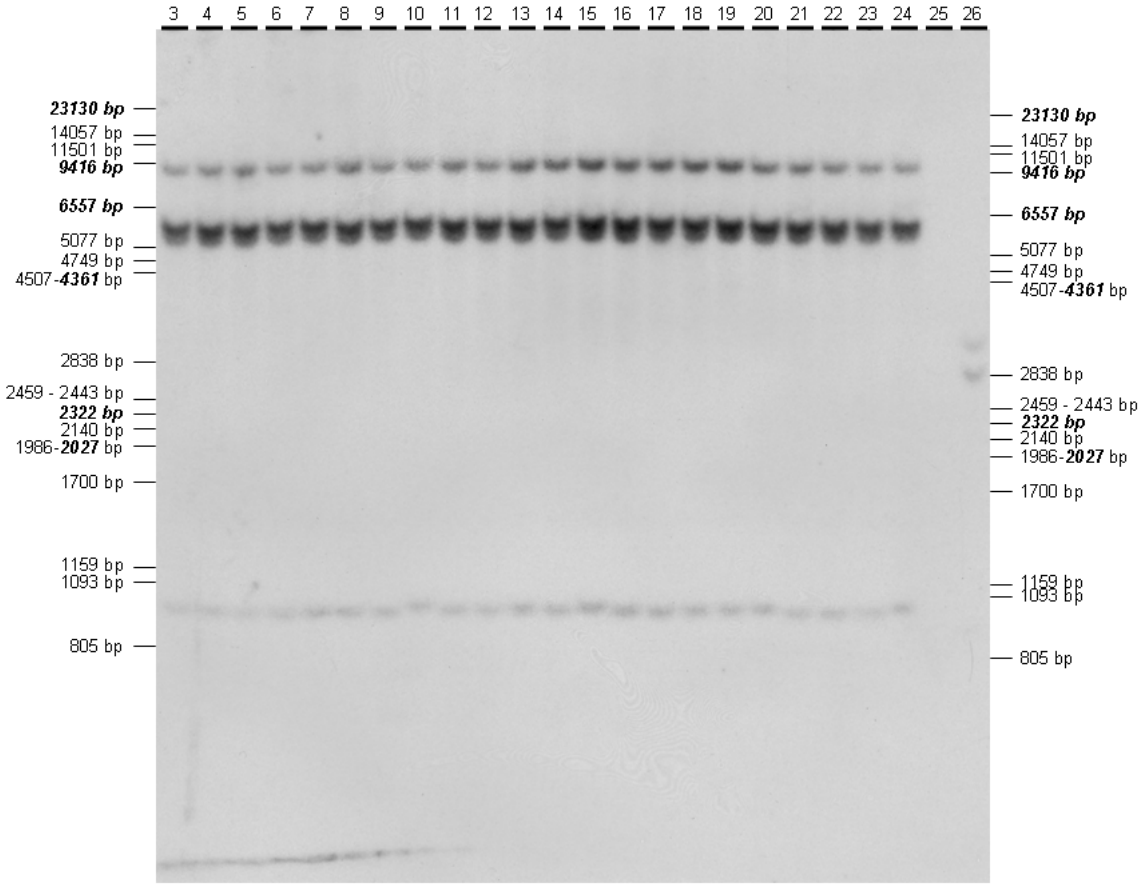

Figure 16: Demonstration of the stability of FG72 – seed lot FG72a-T2 till FG72d-T2 (Generations: T2, Probe: T-DNA)

Gel: NS-TV/BA00627/01
Probe: T-DNA
Film: HYB-TV/BA00627/01/03-F1

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot FG72a-T2 till FG72d-T2) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 239 - *Hin*dIII
Lane 4: FG72 plant 251 - *Hin*dIII
Lane 5: FG72 plant 273 - *Hin*dIII
Lane 6: FG72 plant 274 - *Hin*dIII
Lane 7: FG72 plant 275 - *Hin*dIII
Lane 8: FG72 plant 276 - *Hin*dIII
Lane 9: FG72 plant 277 - *Hin*dIII
Lane 10: FG72 plant 278 - *Hin*dIII
Lane 11: FG72 plant 279 - *Hin*dIII
Lane 12: FG72 plant 280 - *Hin*dIII
Lane 13: FG72 plant 281 - *Hin*dIII
Lane 14: FG72 plant 282 - *Hin*dIII
Lane 15: FG72 plant 283 - *Hin*dIII
Lane 16: FG72 plant 284 - *Hin*dIII
Lane 17: FG72 plant 285 - *Hin*dIII
Lane 18: FG72 plant 286 - *Hin*dIII
Lane 19: FG72 plant 287 - *Hin*dIII
Lane 20: FG72 plant 288 - *Hin*dIII
Lane 21: FG72 plant 289 - *Hin*dIII
Lane 22: FG72 plant 290 - *Hin*dIII
Lane 23: FG72 plant 291 - *Hin*dIII
Lane 24: FG72 plant 301 - *Hin*dIII
Lane 25: Non-transgenic variety JACK - *Hin*dIII
Lane 26: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 27: Phage Lambda – *Pst*I digested
Lane 28: Phage Lambda – *Hin*dIII digested

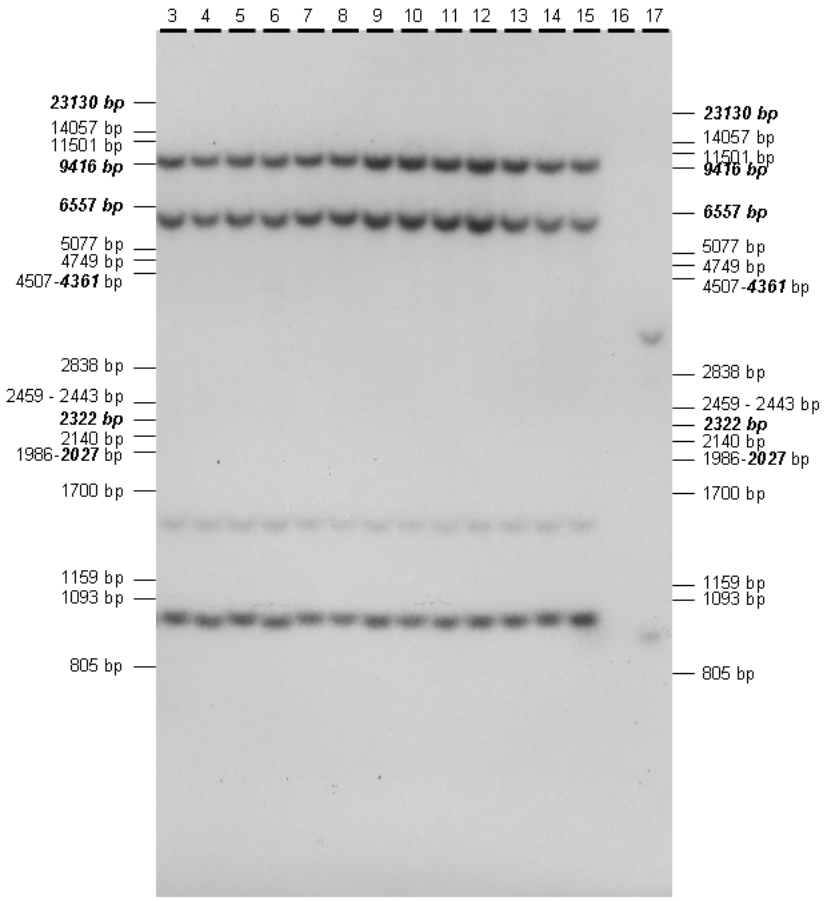

Figure 17: Demonstration of the stability of FG72 – seed lot FG72-x-x-14-5-1-6-x T7 (Generations: T7, Probe: Ph4a748B)

Gel: NS-TV/BA00627/03
Probe: Ph4a748B
Film: HYB-TV/BA00627/03/01-F4

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot FG72-x-x-14-5-1-6-x T7) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the Ph4a748B probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 212 - *Hin*dIII
Lane 4: FG72 plant 213 - *Hin*dIII
Lane 5: FG72 plant 214 - *Hin*dIII
Lane 6: FG72 plant 215 - *Hin*dIII
Lane 7: FG72 plant 216 - *Hin*dIII
Lane 8: FG72 plant 217 - *Hin*dIII
Lane 9: FG72 plant 218 - *Hin*dIII
Lane 10: FG72 plant 219 - *Hin*dIII
Lane 11: FG72 plant 221 - *Hin*dIII
Lane 12: FG72 plant 222 - *Hin*dIII
Lane 13: FG72 plant 223 - *Hin*dIII
Lane 14: FG72 plant 224 - *Hin*dIII
Lane 15: FG72 plant 225 - *Hin*dIII
Lane 16: Non-transgenic variety JACK - *Hin*dIII
Lane 17: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 18: Phage Lambda – *Pst*I digested
Lane 19: Phage Lambda – *Hin*dIII digested

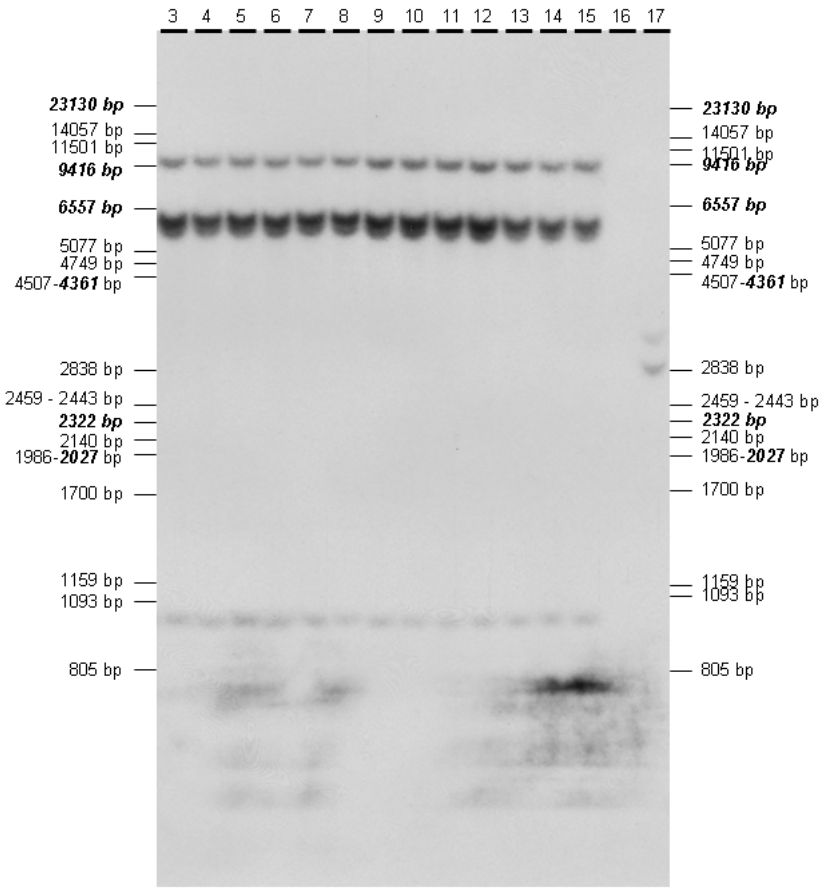

Figure 18: Demonstration of the stability of FG72 – seed lot FG72-x-x-14-5-1-6-x T7 (Generations: T7, Probe: T-DNA)

Gel: NS-TV/BA00627/03
Probe: T-DNA
Film: HYB-TV/BA00627/03/02-F2

Genomic DNA was isolated from *Glycine max* transformation event FG72 plants (seed lot FG72-x-x-14-5-1-6-x T7) and from the non-transgenic counterpart (Wild type Jack). Genomic DNA was digested with *Hin*dIII and probed with the T-DNA probe.

Lane 1: Phage Lambda – *Hin*dIII digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: FG72 plant 212 - *Hin*dIII
Lane 4: FG72 plant 213 - *Hin*dIII
Lane 5: FG72 plant 214 - *Hin*dIII
Lane 6: FG72 plant 215 - *Hin*dIII
Lane 7: FG72 plant 216 - *Hin*dIII
Lane 8: FG72 plant 217 - *Hin*dIII
Lane 9: FG72 plant 218 - *Hin*dIII
Lane 10: FG72 plant 219 - *Hin*dIII
Lane 11: FG72 plant 221 - *Hin*dIII
Lane 12: FG72 plant 222 - *Hin*dIII
Lane 13: FG72 plant 223 - *Hin*dIII
Lane 14: FG72 plant 224 - *Hin*dIII
Lane 15: FG72 plant 225 - *Hin*dIII
Lane 16: Non-transgenic variety JACK - *Hin*dIII
Lane 17: Non-transgenic variety JACK - *Hin*dIII + equimolar amount pSF10 - *Hin*dIII
Lane 18: Phage Lambda – *Pst*I digested
Lane 19: Phage Lambda – *Hin*dIII digested

REFERENCES

No	Doc No	Report No	Author(s), year, title, source, edition, pages
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