Title

Detailed insert characterization of *Glycine max* transformation event FG72 by Southern blot analysis.

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STUDY IDENTIFICATION PAGE

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SUMMARY

Bayer Bioscience N.V. has introduced a *2mepsps* gene construct, encoding tolerance to glyphosate, and a *hppdPf W336* gene construct, encoding tolerance to isoxaflutole, in *Glycine max* by means of direct gene transfer.

For the purpose of this study, genomic DNA was isolated from transgenic plants. Isolated DNA samples were subjected to Southern blot analysis using the different components of the transgenic cassette as well as the complete T-DNA fragment as probes.

According to the model of the FG72 insert organization, the inserted transgenic sequence in *Glycine max* transformation event FG72 consists of 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 at the 3' junction by 158 bases of Ph4a748 promoter sequences.

The obtained hybridization results support this model of the FG72 insert organization.

1. OBJECTIVE

Bayer BioScience N.V. has introduced a *2mepsps* gene construct, encoding tolerance to glyphosate, and a *hppdPf W336* gene construct, encoding tolerance to isoxaflutole, in *Glycine max* by means of direct gene transfer. In this study, the detailed insert characterization of *Glycine max* transformation event FG72 was determined by means of Southern Blot analysis.

2. OVERVIEW OF EXPERIMENTAL DESIGN

Genomic DNA, prepared from transgenic FG72 plants and from wild type *Glycine max* plants (variety JACK), is digested with different restriction enzymes. The resulting DNA fragments are separated by agarose gel-electrophoresis, transferred to a membrane (blot) and sequentially hybridized with different radioactive labeled probes: eight probes containing each single genetic element present in the pSF10 vector used for the transformation, and the complete T-DNA probe.

3. TEST ITEM, REFERENCE ITEMS AND STANDARDS

3.1. Test item

The test item in this study was leaf material taken from plants of *Glycine max* transformation event FG72.

The certified seed lot 32RRMM0497 (CoA n° MDA-09-002) was used to produce the FG72 leaf material. This seed lot was produced by Jan Aelvoet, reference material management, Bayer BioScience N.V.. FG72 plants were grown according to respectively greenhouse protocol 08BAGM0013 and 09BAGM0007. Harvested tissues were directly frozen in liquid nitrogen and transported on dry ice. Upon arrival at the Bayer BioScience N.V. test facility, the frozen leaf material was stored in the ultrafreezer until DNA preparation. Identification of the test item was performed by Molecular Diagnostics Applications – Bayer BioScience N.V. (Identification documents CoA n° MDA-08-009 and MDA-09-010).

3.2. Reference items

During this study, two reference items are used:

3.2.1. Leaf material taken from non transgenic plants of *Glycine max* variety JACK

Non transgenic *Glycine max* plants variety JACK were grown and sampled in the Bayer BioScience N.V. greenhouse (BioScience N.V. – Research Station, Astene, Belgium) by Hans Ronsse according to greenhouse protocol 08BAGM0018. Harvested tissues were directly frozen in liquid nitrogen and transported on dry ice. Upon arrival at the Bayer BioScience N.V. test facility, the frozen leaf material was stored in the ultrafreezer until DNA preparation. Identification of this reference item was performed according to quality standard SQ-STD-GM902 by Molecular Diagnostics Applications – Bayer BioScience NV.

3.2.2. *E. coli* cells containing pSF10 plasmid DNA (Figure 1)

E. coli cells strain DH5α containing the pSF10 plasmid were supplied by Kristel D'hont (Bayer BioScience N.V. – Research, Trait Biology, Gent).

3.3. Standards

3.3.1. Probe templates

Eight probe templates containing each one genetic element of pSF10 (PT015, PT016, PT024 and PT059 to PT063) and the complete T-DNA probe (PT058) were used for hybridization (Table 2, Figure 2). These probe templates are standards.

3.3.2. λ DNA digested with *Pst*I or with *Hin*dIII.

When genomic DNA of the bacteriophage Lambda is digested with the restriction enzyme *Pst*I or *Hin*dIII, restriction fragments of known size are obtained which are used as molecular weight markers.
The λ DNA is supplied by Promega. λ-*Pst*I digested and λ-*Hin*dIII digested are prepared in the Bayer BioScience N.V. Test Facility. The stock solutions and the working solutions of λ-*Pst*I digested and of λ-*Hin*dIII digested are stored in respectively the freezer and the refrigerator.

4. EXPERIMENTAL DESIGN

4.1. Preparation of plasmid DNA

pSF10 and pTEM2 plasmid DNA was prepared using the Wizard® Plus Midipreps DNA Purification System from Promega (SOP BBS 07/18/03).

4.2. Preparation and quantification of plant DNA

Total genomic DNA isolation from FG72 and from the wild type JACK leaf material was performed according to Dellaporta *et al.* (1983) (SOP BBS 07/16/03). DNA quality control and DNA concentration determination was performed by loading a dilution on a 1% TAE agarose gel (SOP BBS 07/25/02). Some genomic DNA samples were pooled and the concentration of these pooled DNA samples was determined mathematically, based on the concentration and the volume of the individual samples that are pooled.

4.3. Preparation of probe templates

All information about the probe templates, including references to the SOPs used for the preparation and purification of the probe templates is listed in Table 2. Table 1 contains the primer sequences.

Probe templates PT015-1, PT016-1, PT024-2 and PT059-1 till PT063-1 were prepared by means of PCR amplification. Probe templates PT015-1, PT016-1 and PT062-1 were prepared with plasmid pTEM2 as template DNA. These probe templates are located in a region of pSF10 that is completely identical to plasmid pTEM2. Other probe templates were prepared with plasmid pSF10 as template DNA. Exact PCR conditions are described in Appendix 2.

In order to prepare a sufficient amount of probe template, at least 30 identical PCR reactions were performed for each probe template preparation. After amplification, all identical PCR reactions were pooled and the volume of this pool was reduced by means of evaporation. The pooled and evaporated samples were loaded on an agarose gel and the fragments of the correct size for each probe were cut out of the gel and purified using the QIAquick Gel Extraction Kit (QIAgen).

Probe template PT058-4 was prepared by means of *Sac*I/*Sma*I restriction digestion of pSF10. 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 concentration of the probe templates was determined by comparing band intensities of the purified probe templates with band intensities of Low and/or High DNA Mass ladder (Invitrogen) after agarose gel electrophoresis and GeneTools analysis according to SOP BBS 07/42/02.

4.4. Restriction digests of total genomic DNA

All restriction digests were performed according to SOP BBS 07/20/03.

Genomic DNA prepared from FG72 plants was submitted to digestion with 10 different restriction enzymes. Each aliquot was digested overnight with one of the following enzymes: *Hinc*II, *Sac*I, *Hind*III, *Bsp*HI, *Apa*I, *Stu*I, *Nco*I, *Sca*I, *Eco*RI and *Bsu36*I. Genomic DNA prepared from wild type plants was digested with the *Hind*III restriction enzyme.

4.5. Restriction digest of pSF10 plasmid DNA

Restriction digestions of pSF10 DNA with *Hind*III were performed according to SOP BBS 07/20/03. Completion of the restriction digestion was confirmed by loading an appropriate amount on a 1% TAE agarose gel (SOP BBS 07/25/02).

4.6. Concentration determination of the digested samples

The concentration of each (pooled) digested genomic DNA sample and of the (pooled) digested pSF10 plasmid DNA was determined by means of the PicoGreen® method according to SOP BBS 07/19/03. The concentrations of all samples that are loaded on the same gel (FG72 genomic DNA, WT genomic DNA and digested pSF10 plasmid DNA) were determined in the same measurement.

4.7. Gel electrophoresis of digested DNA

The agarose gels were prepared according to SOP BBS 07/26/06.

Each gel used for the Southern blot analysis contained:

- A DNA negative control: digested genomic DNA prepared from a WT plant. The negative control was used to confirm the absence of background hybridization;
- DNA positive control: digested genomic DNA of a WT plant supplemented with an equimolar (NG/09-005/18 till NG/09-005/20) or half of an equimolar (NG/09-005/01 till NG/09-005/17) amount of digested transforming plasmid DNA (*Glycine max* genome size: 2.2×10^9 bp (Arumuganathan K. and Earle E.D. (1991)). This control was used to demonstrate that the hybridizations were performed under conditions allowing hybridization of the probes with the target sequences;
- λ-*Pst*I digested and λ-*Hin*dIII digested as molecular weight markers. These molecular weight markers covered the appropriate size range of the hybridization fragments that were expected to be detected by means of Southern blot analysis.

4.8. Loading sequence of the gels

The loading sequence of gels was as follows:

Lane 1: Phage Lambda – *Pst*I digested
Lane 2: Phage Lambda – *Hin*dIII digested
Lane 3: Empty lane
Lane 4: *Glycine max* elite event FG72 – *Hinc*II digested
Lane 5: *Glycine max* elite event FG72 – *Sac*I digested
Lane 6: *Glycine max* elite event FG72 – *Hin*dIII digested
Lane 7: *Glycine max* elite event FG72 – *Bsp*HI digested
Lane 8: *Glycine max* elite event FG72 – *Apa*I digested
Lane 9: *Glycine max* elite event FG72 – *Stu*I, digested
Lane 10: *Glycine max* elite event FG72 – *Nco*I digested
Lane 11: *Glycine max* elite event FG72 – *Sca*I digested
Lane 12: *Glycine max* elite event FG72 – *Eco*RI digested
Lane 13: *Glycine max* elite event FG72 – *Bsu36*I digested

Lane 14: *Glycine max* wild type variety JACK – *Eco*RV digested
Lane 15: *Glycine max* wild type variety JACK – *Hin*dIII digested + (half of) an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hin*dIII digested

After electrophoresis, images were taken using the G:BOX BioImaging system (Syngene) (SOP BBS 04/77/01).

4.9. Blotting of the restriction fragments

Transfer of the separated DNA fragments from the agarose gel to a Hybond-XL membrane (GE Healthcare) was performed according to SOP BBS 07/30/02 (Alkali Blotting, M/09-005/01 and M/09-005/05 till M/09-005/8) or to a Hybond N^+ membrane (GE Healthcare) according to SOP BBS 07/32/00 (Neutral Blotting, M/09-005/18 till M/09-005/20).

To check that the DNA was completely transferred to the membranes, a photo was taken from the gels after blotting (SOP BBS 04/77/01).

4.10. Hybridization

The probe templates were 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) (SOP BBS 07/33/03).

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.

Hybridization and washing steps were performed according to Sambrook *et al*. (SOP BBS 07/34/03).

Visualisation of the hybridizing fragments is achieved after exposure of a BioMax MR film, according to SOP BBS 07/35/02. Films were developed using the Fuji Medical Film Processor FPM100A (SOP BBS 04/73/01).

Probes were removed from the membranes by stripping (BBS 07/36/02).

4.11. Determination of the hybridization fragment sizes

Lengths of the hybridization fragments were determined using the GeneTools software according to SOP BBS 07/42/02. This method was validated by determination of the fragment sizes of the plasmid control. The data were acceptable since the determined fragment sizes differed less than 10% from the expected values.

The mean from the different values obtained for the same fragment was calculated and this value was rounded to the nearest tens. The individual fragment sizes were all within 10% of the mean value.

In case the size of a fragment is known (e.g. for internal fragments), this size is also reported as the obtained fragment size if the rounded mean value differs less than 10% from the expected value. In case the exact size of a fragment is not known, the rounded mean value is reported as obtained value. Size determination for fragments of more than 14 Kb is not possible with this experimental setup. These fragments are noted as '>14kb'.

5. RESULTS AND DISCUSSION

Each membrane used for the analysis contained one DNA negative control, in which the template DNA was genomic DNA prepared from a non-transgenic JACK plant. This negative control showed no hybridization with any of the probes used (Figure 3 to Figure 11, lane 14), confirming the absence of background hybridization. Similarly, each membrane contained one positive control: digested genomic DNA prepared from a non-transgenic JACK plant, supplemented with (half of) an equimolar amount of the transforming plasmid pSF10 digested with *Hin*dIII. With each of the probes used, *Hin*dIII fragments of expected size were detected (Figure 3 to Figure 11, lane 15). This indicates that the Southern blot analysis was performed under appropriate conditions allowing specific hybridization of the probes used on target sequences.

The inserted transgenic sequence in *Glycine max* transformation event FG72 consists of 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 at the 3' junction by 158 bases of Ph4a748 promoter sequences. Figure 2 shows a schematic presentation of this model with indication of the restriction enzymes and probes used and the expected hybridization fragments.

The expected and obtained hybridization fragments for each of the 10 different restriction enzymes are listed in Table 3. The obtained hybridization results are presented in Figure 3 to Figure 11. Hybridization results support the model of the FG72 insert organization as described above and as depicted in Figure 2.

A number of fragments did run atypically on the agarose gel. This is probably caused by secondary DNA structures. A number of expected fragments could not be visualized, due to a small overlap between the fragments and the T-DNA probe and/or to the ratio fragment length/probe length.

*Apa*I digested FG72 genomic DNA shows after hybridization with all probes an additional fragment of 8570 bp (Table 3). This fragment is also visible after hybridization with the 3' nos probe (PT024-2), indicating that it comes from incomplete digestion of the *Apa*I restriction site between the internal 558 bp fragment and the 7900 bp 3' integration fragment. Hybridization results of FG72 genomic DNA digested with *Bsu*36I show after hybridization with probe templates PT059-1 and PT062-1 the presence of very weak unexpected fragments of 11590 bp and >14 kb (Figure 7 lane 13, Figure 8 lane 13, Table 3), coming from incomplete digestion.

6. CONCLUSION

Genomic DNA of *Glycine max* transformation event FG72 was digested with ten different restriction enzymes and hybridized with different probe templates, each containing a component of the transforming gene cassette, and the complete T-DNA probe.

The hybridization results are in line with the model of the FG72 insert organization. According to this model, the inserted transgenic sequence in *Glycine max* transformation event FG72 consists of 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 transformation, a genomic region translocated to a new position, which is joined by 158 bases of Ph4a748 promoter sequence at the 3' junction.

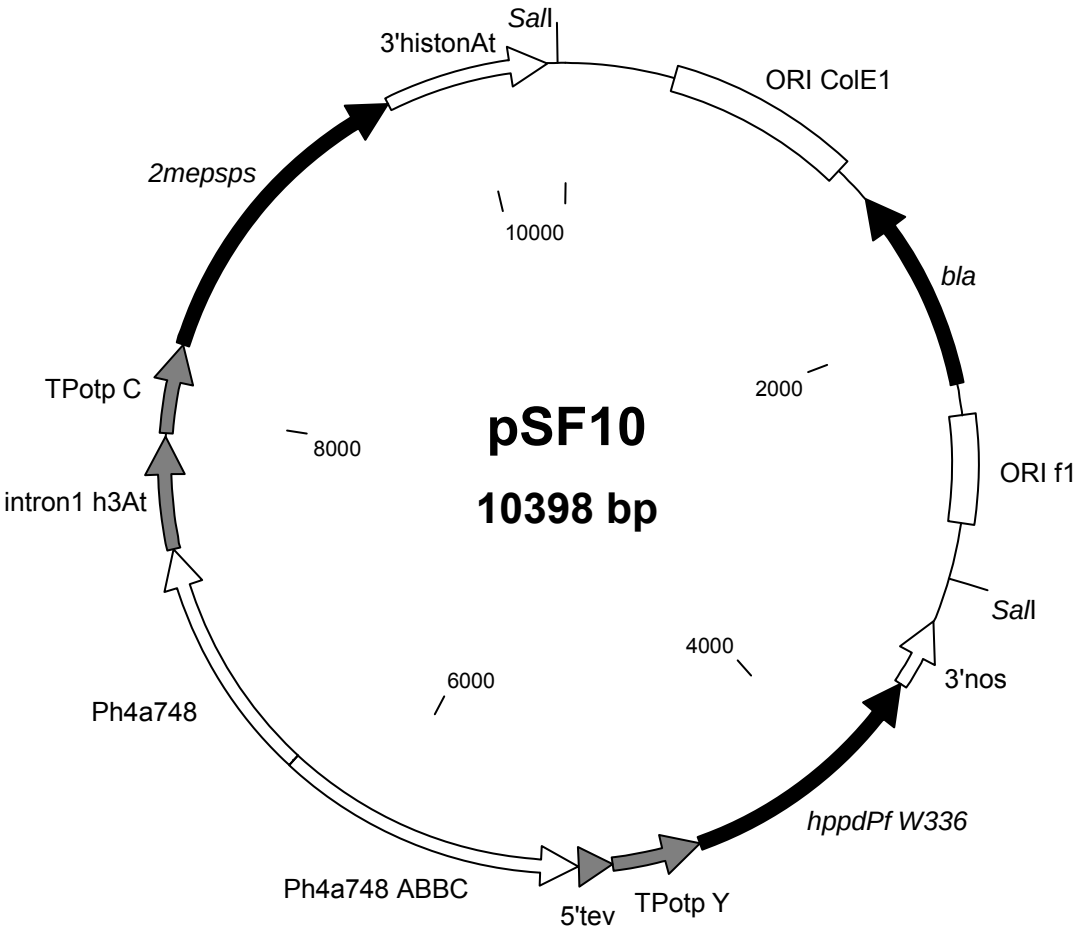

Figure 1. map of vector pSF10

Table 1. Information on primers used for probe template preparation

Primer pair	Primer sequence (5' → 3')	Primer position in pSF10 (bp)	Primer position in pTEM2 (bp)
SMP084	Critical Confidential Information removed	8309 → 8329	1934 → 1954
MLD090		9641 ← 9659	3266 ← 3284
DPA207		9614 → 9633	3239 → 3258
SMP086		10348 ← 10366	3973 ← 3991
MDB355		3265 → 3285	/
MDB055		3460 ← 3478	/
SMP131		6491 → 6510	116 → 135
KM008		7428 ← 7449	1053 ← 1074
MLD148		7446 → 7465	1071 → 1090
SMP052		7933 ← 7952	1558 ← 1577
MLD123		4650 → 4676	/
STV129		5086 ← 5109	/
JDB018		6866 → 6891 5997← 6022*	491 → 516
JDB019		7269 ← 7295 5260 → 5286*	894 ← 920
SMP083		3558 → 3581	/
SMP082		4592 ← 4612	/

To avoid confusion between bases, a lower-case 'g' is used to clearly differentiate between 'g' and 'C'
*primers JDB018 and JDB019 have 2 possible priming sites in plasmid pSF10. The priming sites indicated with * are not needed for the probe template preparation

Table 2. Information on probe templates used

Probe template ID	Template DNA	Description	Primer pair / Restriction digest	Size probe template	Conc (ng/µL)	Prepared in study	SOP n° PCR/Digest	SOP n° evaporation	SOP n° agarose gel electro-phoresis	SOP n° gel extraction
PT015-1	pTEM2 *	*2mepsps*	SMP084-MLD090	1351 bp	30	BBS06-001	BBS 07/21/00	BBS 04/29/00	BBS 07/25/00	BBS 07/27/01
PT016-1	pTEM2 *	3'histonAt	DPA207-SMP086	753 bp	40	BBS06-001	BBS 07/21/00	BBS 04/29/00	BBS 07/25/00	BBS 07/27/01
PT024-2	pSF10	3'nos	MDB355-MDB055	214 bp	140	BBS09-005	BBS 07/21/03	BBS 04/29/02	BBS 07/25/02	BBS 07/27/04
PT058-4	pSF10	T-DNA probe	*Sac*I/*Sma*I	7204 bp	34	BBS09-003	BBS 07/20/03	/	BBS 07/25/02	BBS 07/27/05
PT059-1	pSF10	Ph4a748	SMP131-KM008	959 bp	30	BBS09-005	BBS 07/21/03	BBS 04/29/02	BBS 07/25/02	BBS 07/27/04
PT060-1	pSF10	Intron1 h3At	MLD148-SMP052	507 bp	40	BBS09-005	BBS 07/21/03	BBS 04/29/02	BBS 07/25/02	BBS 07/27/04
PT061-1	pSF10	5'tev + TPotp Y	MLD123-STV129	460 bp	50	BBS09-005	BBS 07/21/03	BBS 04/29/02	BBS 07/25/02	BBS 07/27/04
PT062-1	pTEM2 *	Ph4a748B	LDB018-JDB019	430 bp	150	BBS09-005	BBS 07/21/03	BBS 04/29/02	BBS 07/25/02	BBS 07/27/04
PT063-1	pSF10	*hppdPf W336*	SMP083-SMP082	1055 bp	160	BBS09-005	BBS 07/21/03	BBS 04/29/02	BBS 07/25/02	BBS 07/27/04

* Probe templates prepared with pTEM2 as template DNA are located in a plasmid region of pTEM2 that is completely identical to plasmid pSF10

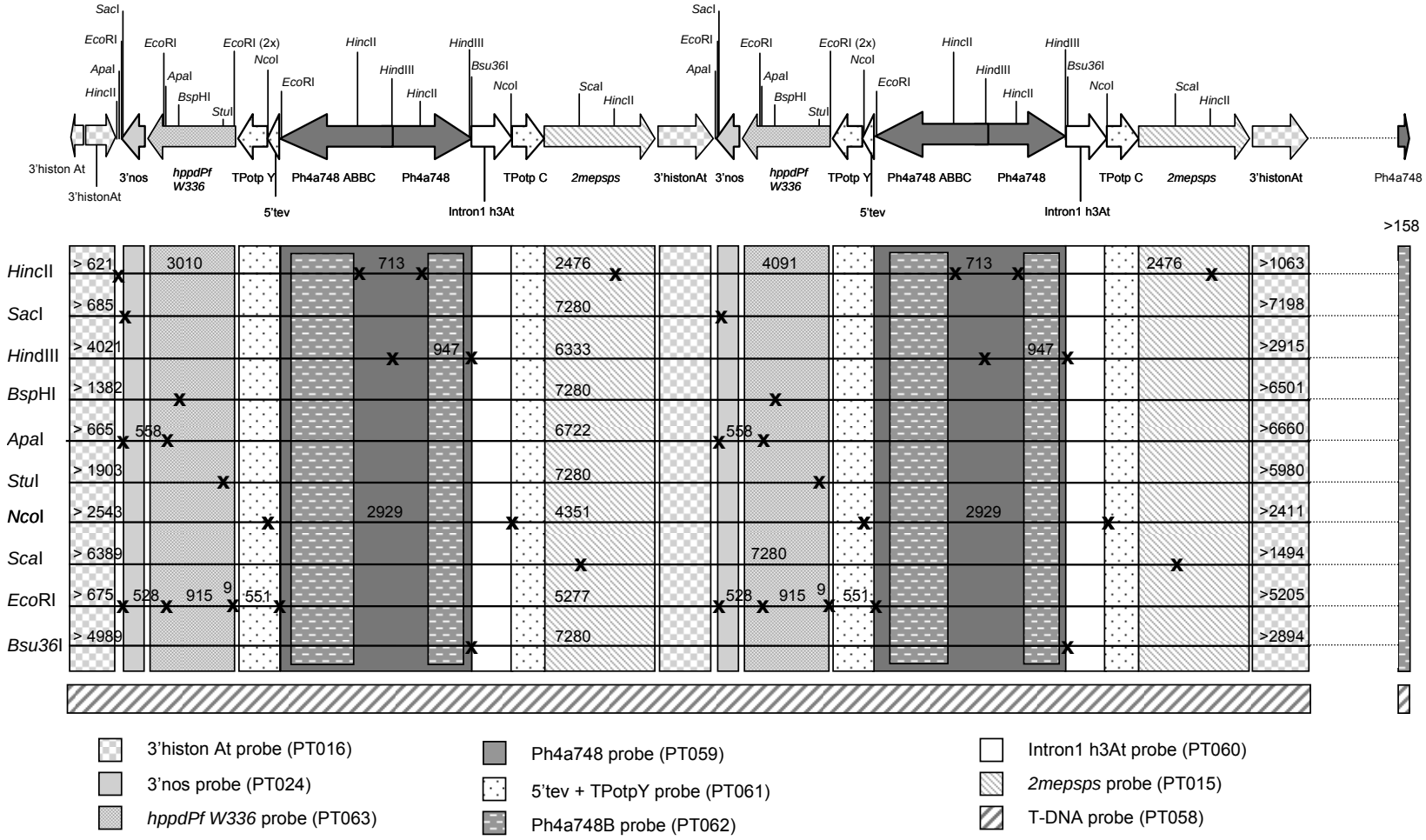

Figure 2. Schematic drawing of the FG72 inserted transgenic sequences with indication of the restriction enzymes and probes used and expected fragment lengths.

Table 3. Expected and obtained hybridization fragments

Digest	Description	Expected fragment sizes (bp)	Obtained fragment sizes (bp)	PT016-1: 3'histonAt		PT024-2: 3'nos		PT063-1: hppdPf W336		PT061-1: 5'tev+TPotp Y		PT059-1: Ph4a748		PT062-1: Ph4a748B		PT060-1: Intron1 h3At		PT015-1: 2mepsps		PT058-4: T-DNA probe	
				Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.
HincII	5' integration fr.	> 621	5250	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	No[a]
	internal fr.	3010	3010	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	internal fr.	4091	4091	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes
	internal fr.	713	713	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	No	No	No	Yes	No[a]
	internal fr.	2476	2476	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' integration fr.	> 1063	1130	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	1300	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No[a]
SacI	5' integration fr.	> 685	6060	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes, weak
	internal fr.	7280	7280	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' integration fr.	> 7198	>14 kb	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	>14 kb	No	No	No	No	No	No	No	No	Yes	No[a]	Yes	Yes	No	No	No	No	Yes	No[a]
HindIII	5' integration fr.	> 4021	9550	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	internal fr.	6333	6333	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	internal fr.	947	947	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	3' integration fr.	> 2915	5500	Yes	Yes	No	No	No	No	Yes	Yes	Yes	No[a]	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	1480	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No[a]
BspHI	5' integration fr.	> 1382	3200	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes
	internal fr.	7280	7280	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' integration fr.	> 6501	7480	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	4260	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No[a]

Digest	Description	Expected fragment sizes (bp)	Obtained fragment sizes (bp)	PT016-1: 3'histonAt		PT024-2: 3'nos		PT063-1: hppdPf W336		PT061-1: 5'tev+TPotp Y		PT059-1: Ph4a748		PT062-1: Ph4a748B		PT060-1: Intron1 h3At		PT015-1: 2mepsps		PT058-4: T-DNA probe	
				Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.
ApaI	5' integration fr.	> 665	10760	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes, weak
	internal fr.	6722	6722	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	internal fr.	558	558	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes
	3' integration fr.	> 6660	7900	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	Partial fr.	/	8570	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes	No	Yes
	3' junction translocation	> 158	>14 kb	No	No	No	No	No	No	No	No	Yes	No[a]	Yes	Yes, weak	No	No	No	No	Yes	No[a]
StuI	5' integration fr.	> 1903	4710	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes
	internal fr.	7280	7280	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' integration fr.	> 5980	6210	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	>14 kb	No	No	No	No	No	No	No	No	Yes	No[a]	Yes	Yes, weak	No	No	No	No	Yes	No[a]
NcoI	5' integration fr.	> 2543	7660	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	Yes	Yes
	internal fr.	2929	2929	No	No	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes
	internal fr.	4351	4351	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No[a]	Yes	Yes	Yes	Yes
	3' integration fr.	> 2411	10270	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	No[a]	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	>14 kb	No	No	No	No	No	No	No	No	Yes	Yes, weak	Yes	Yes	No	No	No	No	Yes	No[a]
ScaI	5' integration fr.	> 6389	9900	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	internal fr.	7280	7280	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' integration fr.	> 1494	4730	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	11430	No	No	No	No	No	No	No	No	Yes	No[a]	Yes	Yes	No	No	No	No	Yes	No[a]
EcoRI	5' integration fr.	> 675	5110[d]	Yes	Yes	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Yes	Yes
	internal fr.	5277	5277[d]	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Digest	Description	Expected fragment sizes (bp)	Obtained fragment sizes (bp)	PT016-1: 3'histonAt		PT024-2: 3'nos		PT063-1: hppdPf W336		PT061-1: 5'tev+TPotp Y		PT059-1: Ph4a748		PT062-1: Ph4a748B		PT060-1: Intron1 h3At		PT015-1: 2mepsps		PT058-4: T-DNA probe	
				Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.	Exp.	Obt.
	internal fr.	528[c]	528[c]	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes
	internal fr.	915	915	No	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No	No	No	Yes	Yes
	internal fr.	9[b]	/	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	internal fr.	551[c]	551[c]	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No	No	Yes	Yes
	3' integration fr.	> 5205	9610	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	4670	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No[a]
Bsu36I	5' integration fr.	> 4989	7650[e]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	internal fr.	7280	7280[e]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
	3' integration fr.	> 2894	10350	Yes	Yes	No	No	No	No	Yes	Yes	No	No	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	3' junction translocation	> 158	7670[e]	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	Partial fr.	/	11590	No	No	No	No	No	No	No	No	No	Yes, weak	No	Yes, weak	No	No	No	No	No	No
	Partial fr.	/	>14 kb	No	No	No	No	No	No	No	No	No	Yes, weak	No	Yes, weak	No	No	No	No	No	No
WT genomic DNA-HindIII				/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
WT genomic DNA - HindIII + pSF10 - HindIII	Positive control	3420	3420	No	No	Yes	Yes	Yes	Yes	Yes	Yes, weak	Yes	Yes	Yes	Yes	No	No	No	No	Yes	Yes
	Positive control	947	947	No	No	No	No	No	No	No	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No[a]
	Positive control	2961	2961	Yes	Yes	No	No	No	No	Yes	Yes, weak	Yes	No[a]	No	No	Yes	Yes	Yes	Yes	Yes	Yes
	Positive control	3070	/	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
Hybridization ID				H1/09-005/01-F2		H1/09-005/20-F3		H1/09-005/08-F1		H1/09-005/19-F7		H1/09-005/06-F2		H1/09-005/07-F2		H1/09-005/18-F1		H1/09-005/05-F2		H2/09-005/19-F1	

[a] to a small overlap between the fragments and the T-DNA probe and/or to the ratio fragment length/probe length, these expected fragments could not be visualized.
[b] This fragment is too small to be visualized.
[c,d, e] These fragments can appear as a single fragment.

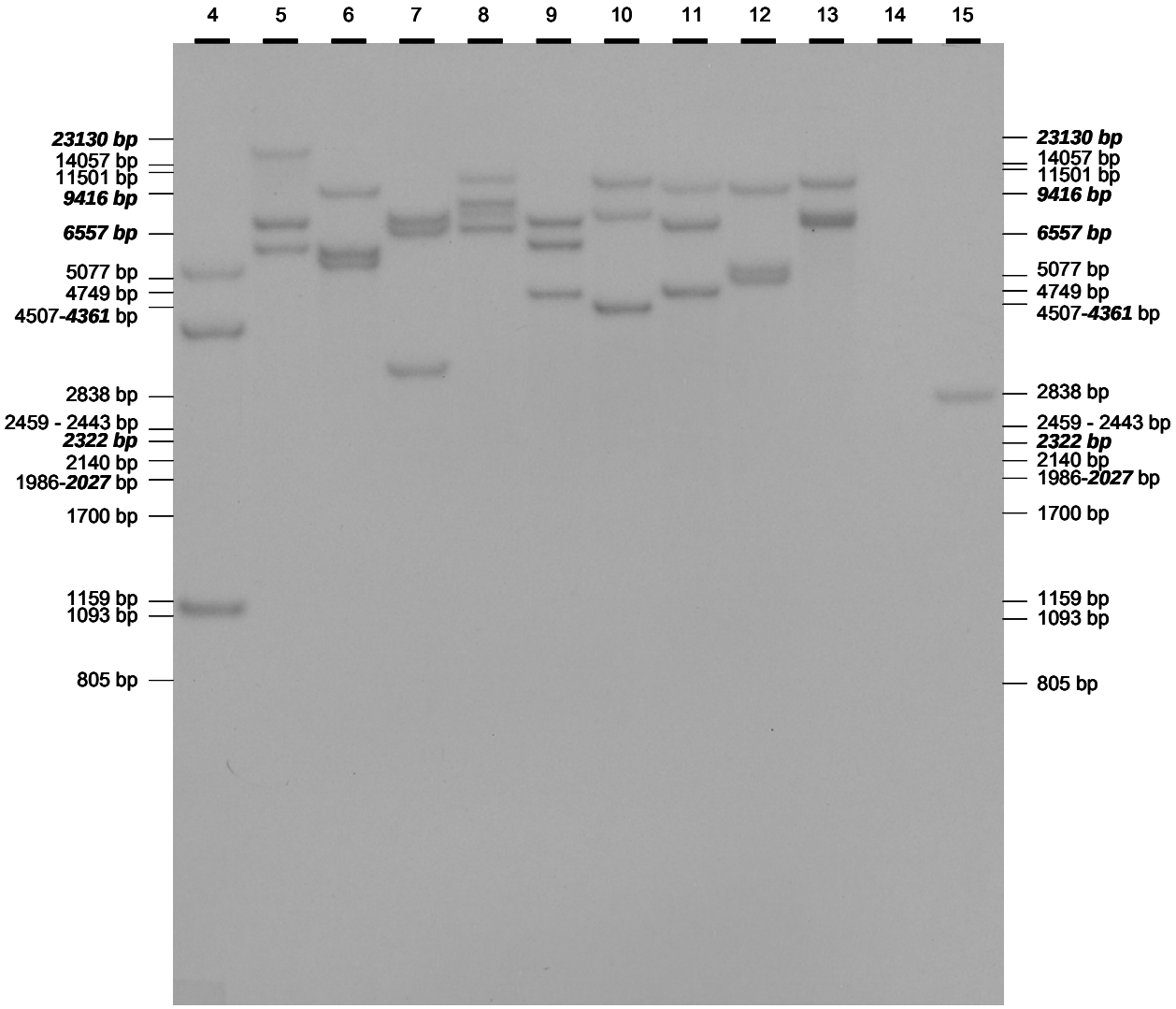

Figure 3. Southern blot analysis FG72 – 3'histonAt probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the 3'histonAt probe (PT016-1: 753 bp DPA207-SMP086 fragment of pTEM2).

Membrane ID: M/09-005/01
Film ID: H1/09-005/01-F2
Probe template ID: PT016-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + half of an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

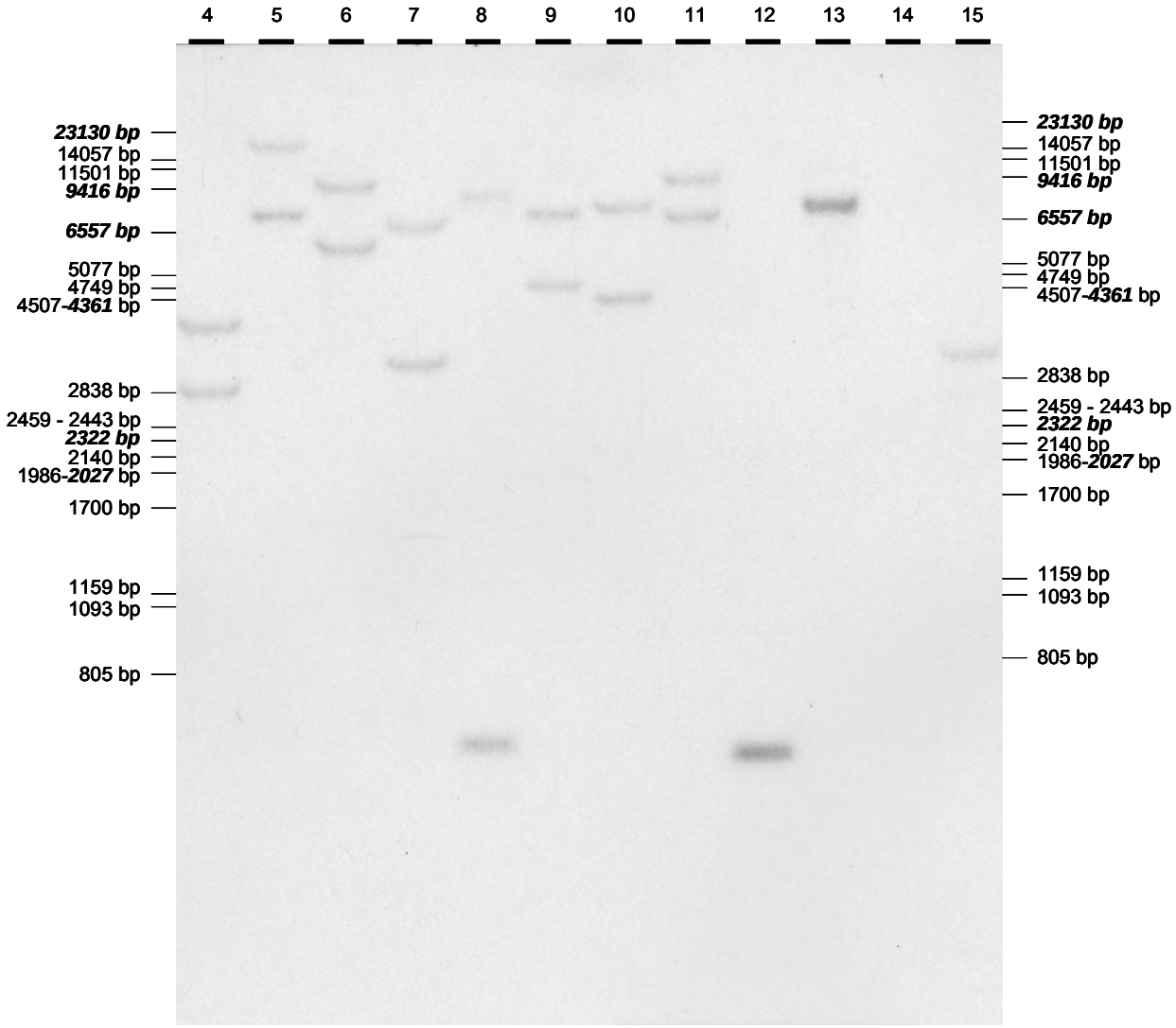

Figure 4. Southern blot analysis FG72 – 3'nos probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the 3'nos probe (PT024-2: 214 bp MDB355-MDB055 fragment of pTDL004).

Membrane ID: M/09-005/20
Film ID: H1/09-005/20-F3
Probe template ID: PT024-2

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

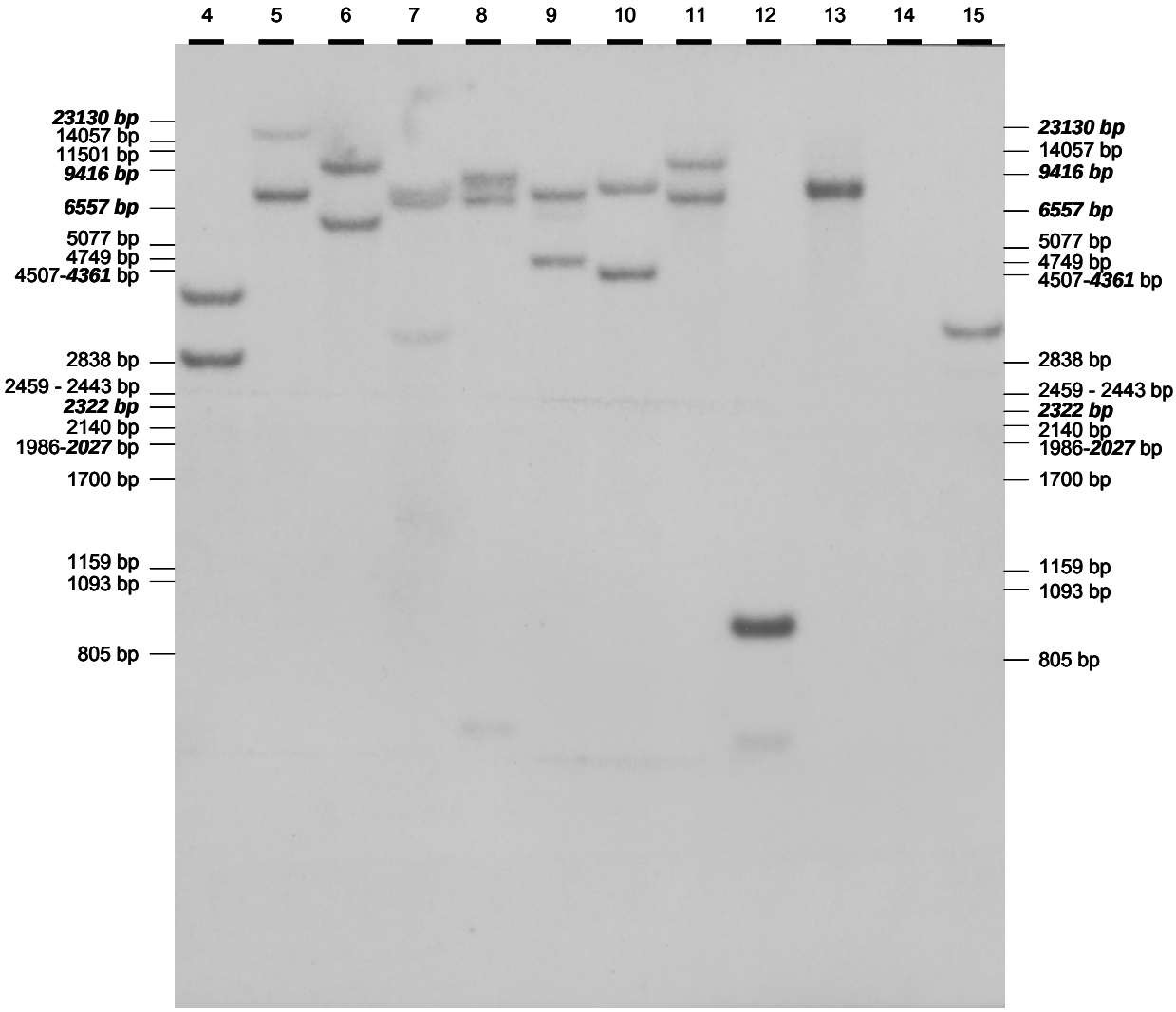

Figure 5. Southern blot analysis FG72 – hppdPf W336 probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the hppdPf W336 probe (PT063-1: 1055 bp SMP083-SMP082 fragment of pSF10).

Membrane ID: M/09-005/08
Film ID: H1/09-005/08-F1
Probe template ID: PT063-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + half of an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

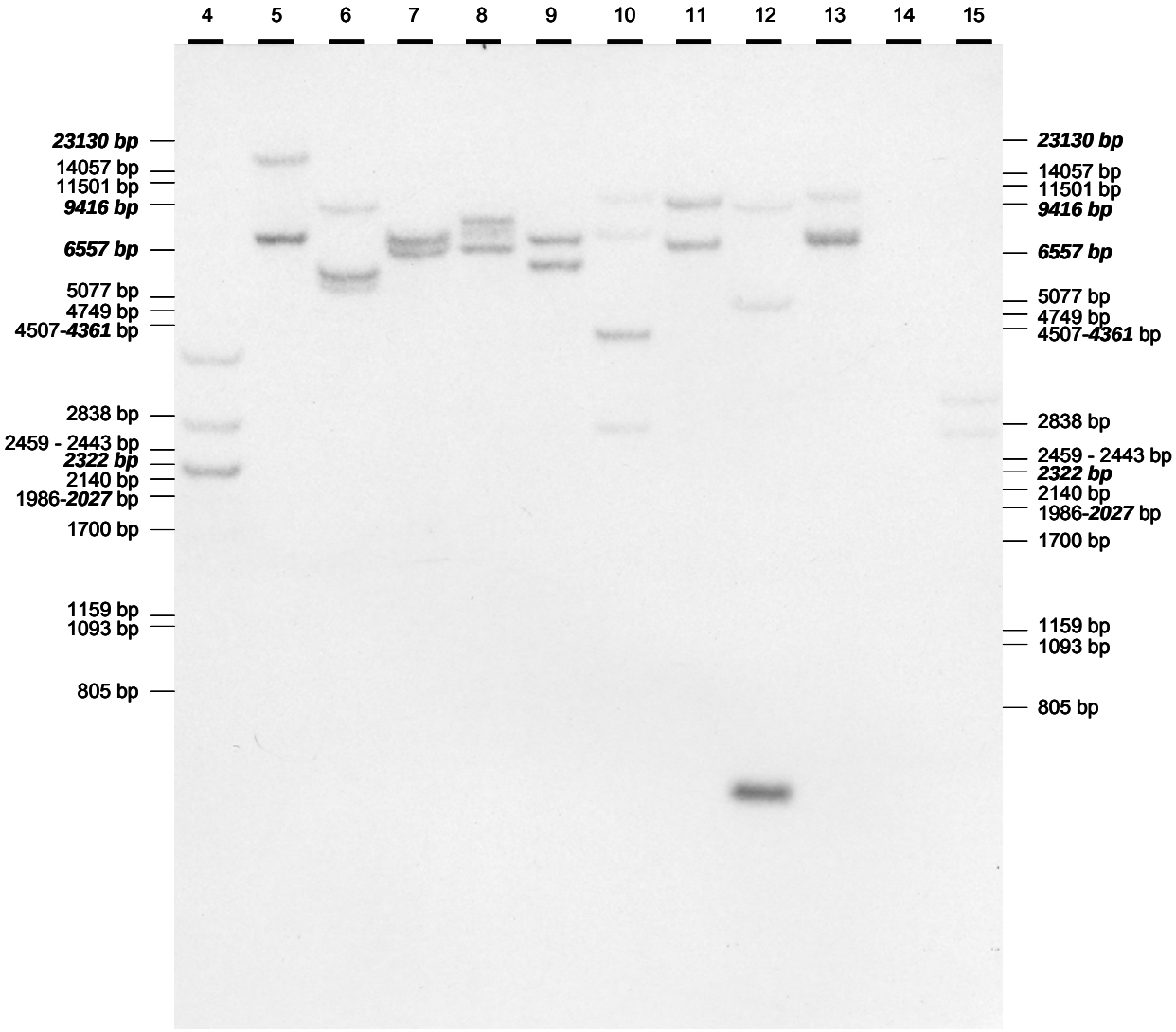

Figure 6. Southern blot analysis FG72 – 5'tev+TPotp Y probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the 5'tev+TPotp Y probe (PT061-1: 460 bp MLD123-STV129 fragment of pSF10).

Membrane ID: M/09-005/19
Film ID: H1/09-005/19-F7
Probe template ID: PT061-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

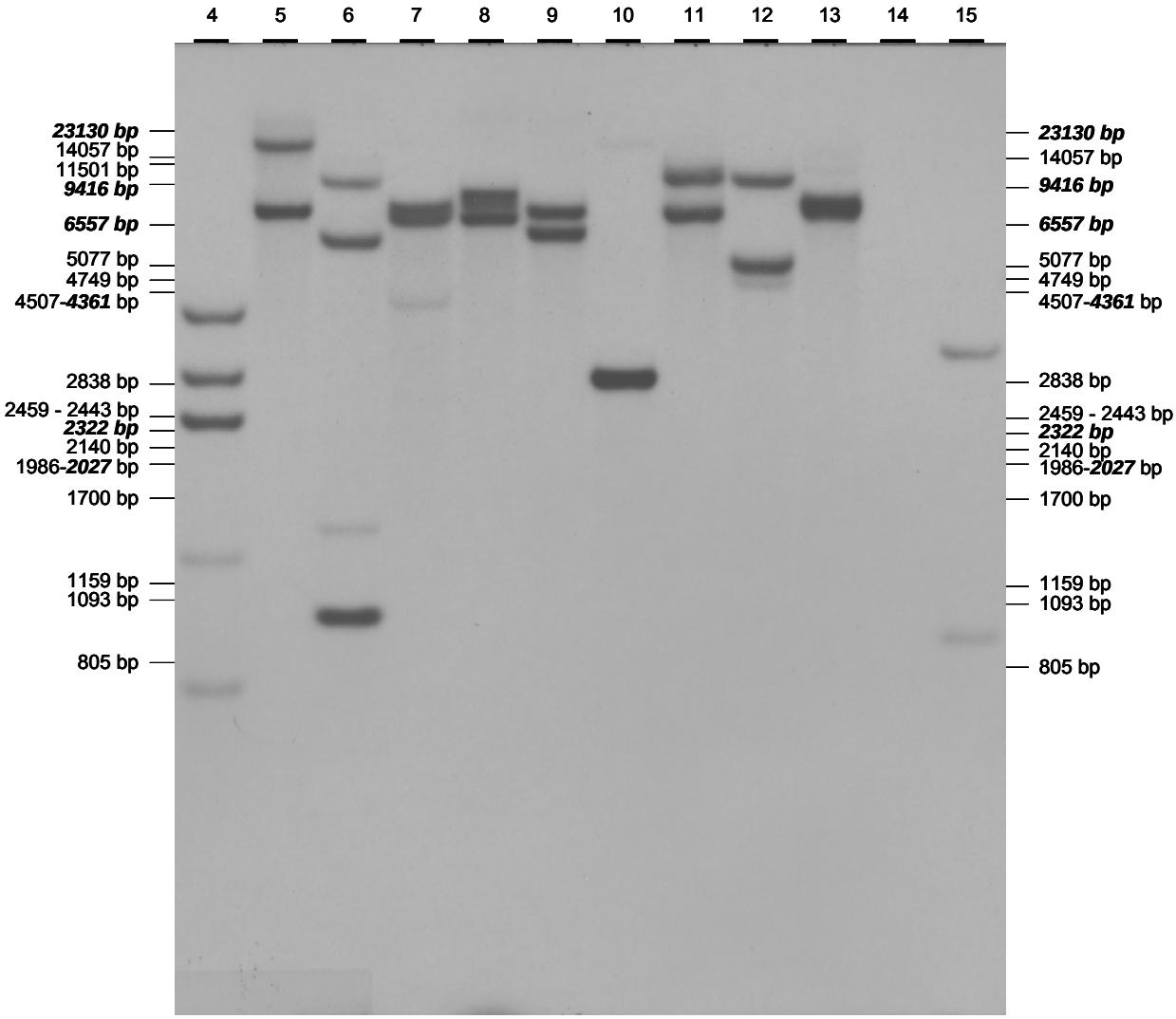

Figure 7. Southern blot analysis FG72 – Ph4a748 probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the Ph4a748 probe (PT059-1: 959 bp SMP131-KM008 fragment of pSF10).

Membrane ID: M/09-005/06
Film ID: H1/09-005/06-F2
Probe template ID: PT059-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + half of an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

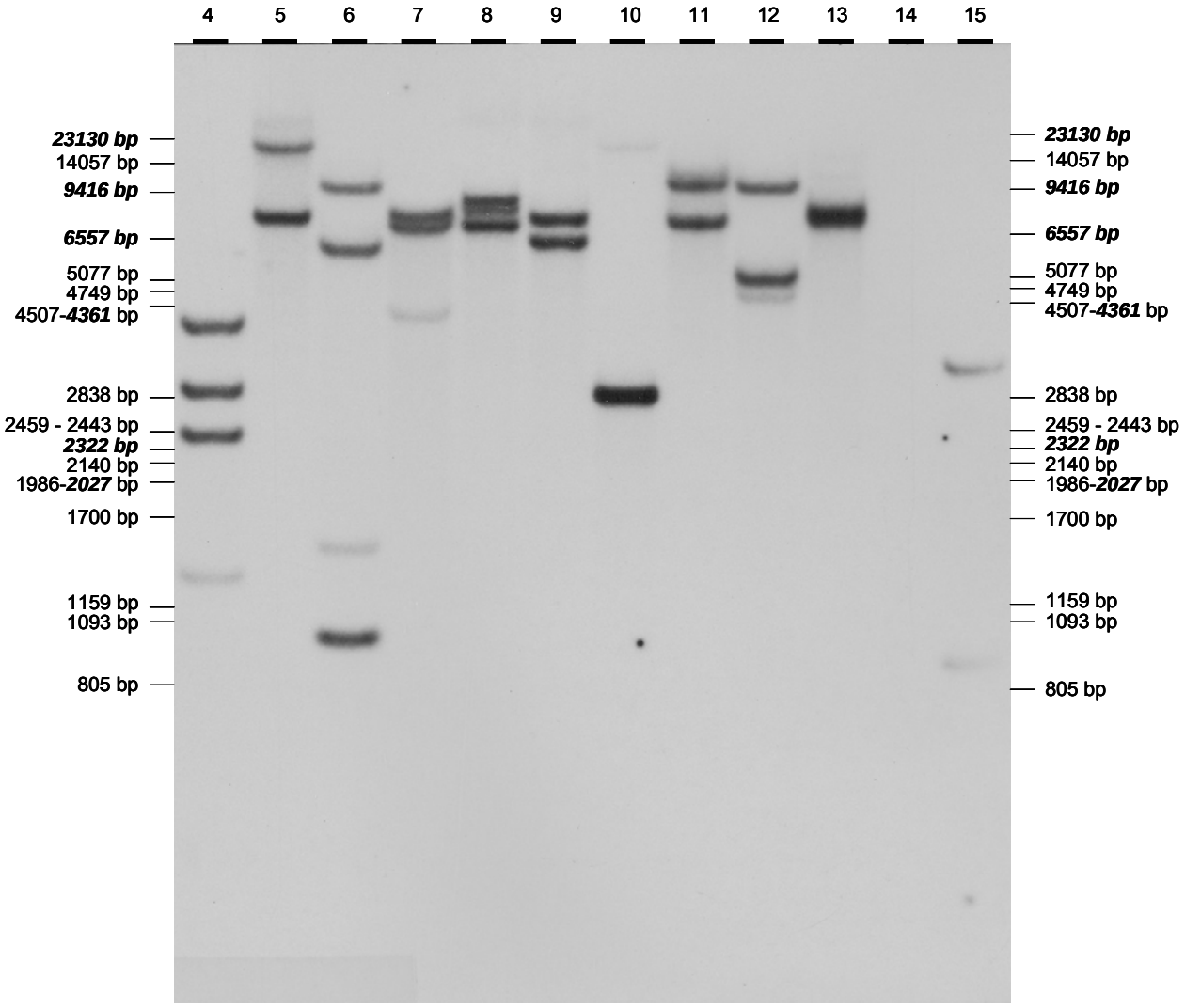

Figure 8. Southern blot analysis FG72 – Ph4a748B probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the Ph4a748B probe (PT062-1: 430 bp JDB018-JDB019 fragment of pTEM2).

Membrane ID: M/09-005/07
Film ID: H1/09-005/07-F2
Probe template ID: PT062-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + half of an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

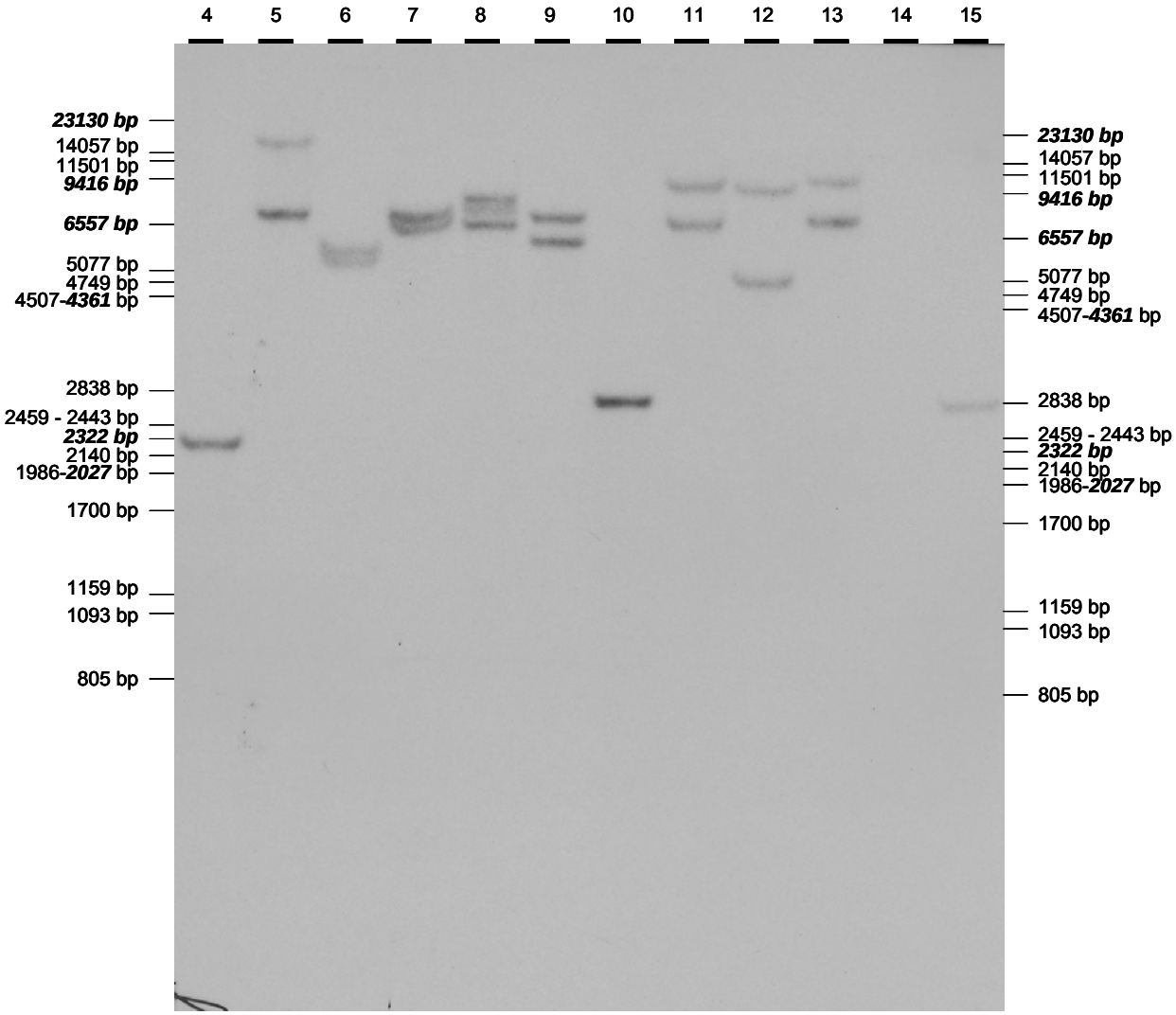

Figure 9. Southern blot analysis FG72 – Intron1 h3At probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the Intron1 h3At probe (PT060-1: 507 bp MLD148-SMP052 fragment of pSF10).

Membrane ID: M/09-005/18
Film ID: H1/09-005/18-F1
Probe template ID: PT060-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

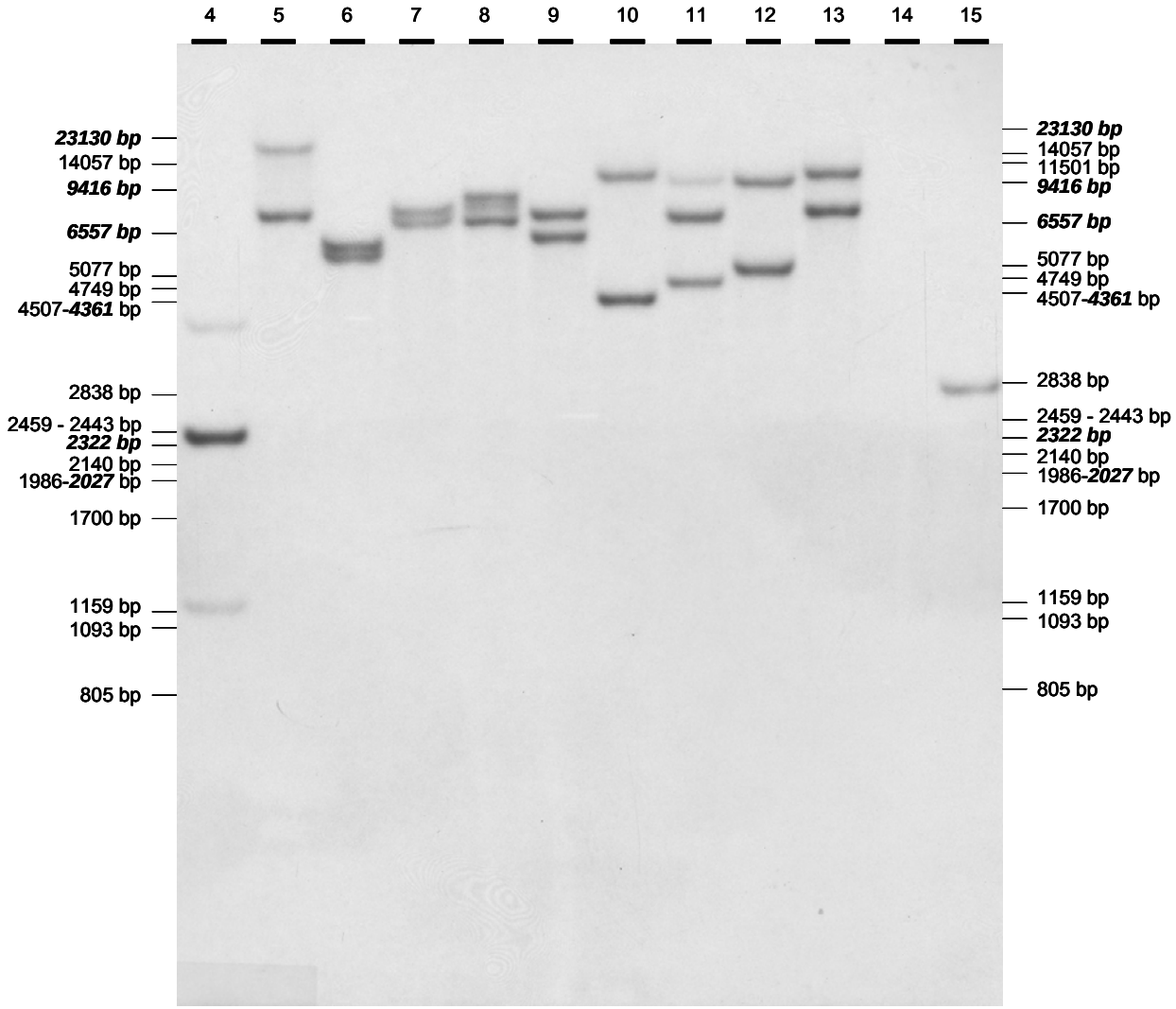

Figure 10. Southern blot analysis FG72 – 2*mepsps* probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the *2mepsps* probe (PT015-1: 1351 bp SMP084-MLD090 fragment of pTEM2).

Membrane ID: M/09-005/05
Film ID: H1/09-005/05-F2
Probe template ID: PT015-1

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + half of an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

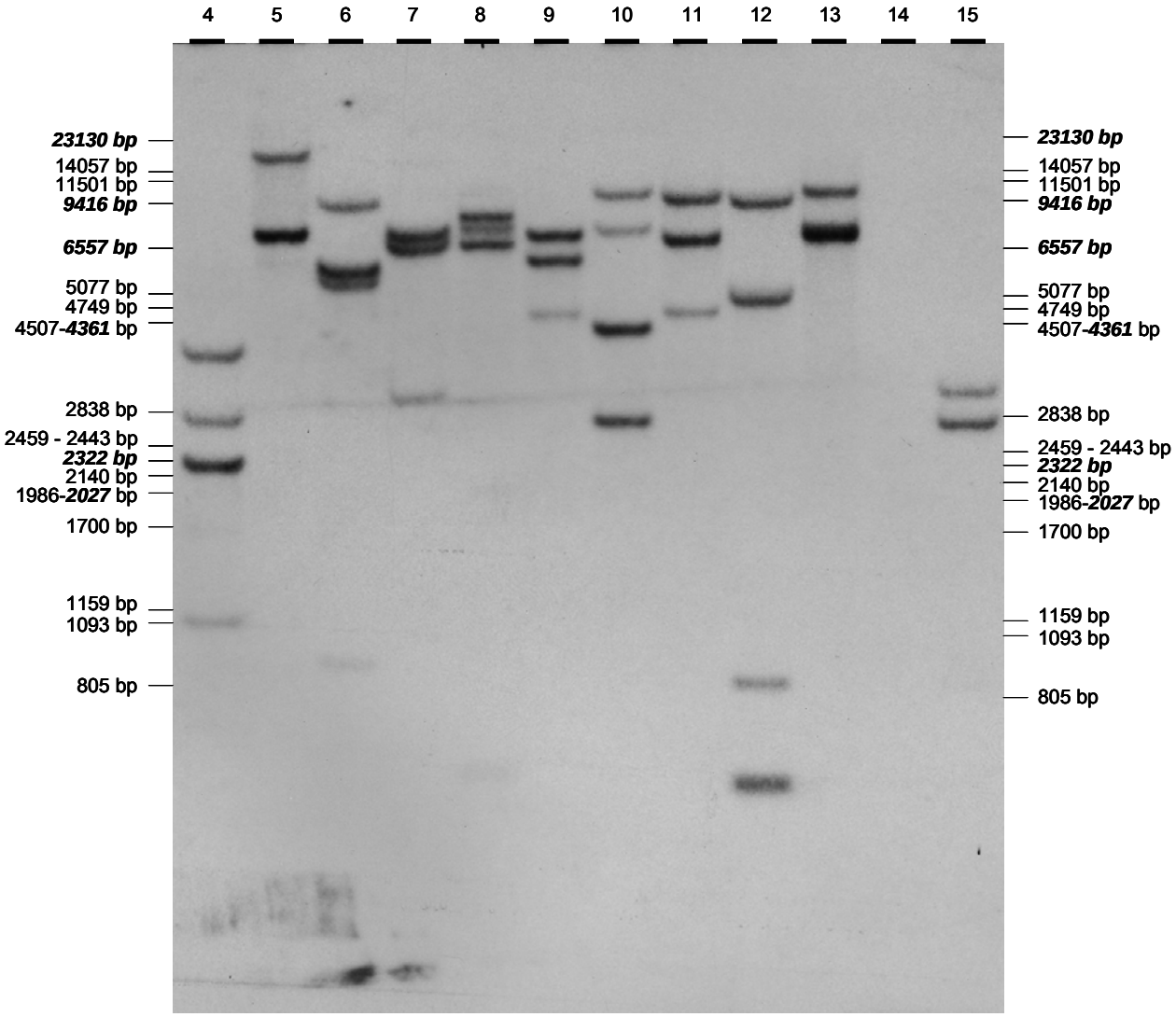

Figure 11. Southern blot analysis FG72 – T-DNA probe
Genomic DNA was isolated from *Glycine max* FG72 plants and from the non-transgenic counterpart Jack. DNA samples (8 µg) were digested with different restriction enzymes and probed with the T-DNA probe (PT058-4: 7204bp SacI/SmaI fragment of pSF10).

Membrane ID: M/09-005/19
Film ID: H2/09-005/19-F1
Probe template ID: PT058-4

Lane 1: Phage Lambda – *Hind*III digested
Lane 2: Phage Lambda – *Pst*I digested
Lane 3: empty
Lane 4: *Glycine max* event FG72 – *Hinc*II digested
Lane 5: *Glycine max* event FG72 – *Sac*I digested
Lane 6: *Glycine max* event FG72 – *Hind*III digested
Lane 7: *Glycine max* event FG72 – *BspH*I digested
Lane 8: *Glycine max* event FG72 – *Apa*I digested
Lane 9: *Glycine max* event FG72 – *Stu*I digested
Lane 10: *Glycine max* event FG72 – *Nco*I digested
Lane 11: *Glycine max* event FG72 – *Sca*I digested
Lane 12: *Glycine max* event FG72 – *Eco*RI digested
Lane 13: *Glycine max* event FG72 – *Bsu36*I digested
Lane 14: *Glycine max* wild type variety Jack – *Hind*III digested
Lane 15: *Glycine max* wild type variety Jack – *Hind*III digested + an equimolar amount of pSF10 – *Hind*III digested
Lane 16: Phage Lambda – *Pst*I digested
Lane 17: Phage Lambda – *Hind*III digested

REFERENCES

No	Doc No	Report No	Author(s), year, title, source, edition, pages
1.	M-232805-01-1		Dellaporta S., Wood J. and Hicks J. (1983). A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter 1(4), 19-21.
2.	- - - - - - -		Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) - Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
3.	M-257571-01-1		Arumuganathan K. and Earle E.D. (1991). Nuclear DNA Content of Some Important Plant Species. Plant Molecular Biology Reporter, 9 (3), 208-218.
4.			Verhaeghe S. (2010). Full DNA sequence of event insert and integration site of *Glycine max* transformation event FG72.

Appendix 1: Overview of the analytical SOPs used in this study

SOP number	Title
BBS 07/16/03	Preparation of genomic DNA according to Dellaporta *et al.*
BBS 07/18/03	Preparation of plasmid DNA from *E. coli* bacterial cells
BBS 07/19/03	Quantification of double stranded DNA - PicoGreen® - method
BBS 07/20/03	Restriction digestion
BBS 07/21/03	PCR – *Taq* DNA polymerase
BBS 07/25/02	Agarose gel electrophoresis – horizontal submarine gel system
BBS 07/26/06	Agarose gel electrophoresis – non-submarine gel system
BBS 07/27/05	DNA gel extraction
BBS 07/30/02	Southern Blot Analysis – Alkali blotting of DNA
BBS 07/32/00	Southern Blot Analysis – Neutral blotting of DNA
BBS 07/33/03	Southern Blot Analysis – [α-^{32}P] labeling of DNA probes
BBS 07/34/04	Southern Blot Analysis – Hybridization
BBS 07/35/02	Southern Blot Hybridization – Autoradiography
BBS 07/36/02	Southern Blot Hybridization – Stripping of membranes
BBS 07/42/02	Fragment analysis using the genetools software

Appendix 2: **PCR conditions used to prepare probe templates**

Mix for probe templates PT062-1 and PT024-2:
Components for a 20 µL reaction:

- 100 pg template DNA
- 2 µL 10x PCR Gold Buffer without $MgCl_2$
- 0.4 µL 10 mM dNTPs
- 1 µL forward primer (10 pmol/µL)
- 1 µL reverse primer (10 pmol/µL)
- 4 µL Betain (5M)
- 1.2 µL $MgCl_2$ (5 mM)
- 0.2 µL Amplitaq® Gold DNA polymerase (5U/µL)
- MQ water up to 20 µL

Thermocycling profile for probe templates PT062-1 and PT024-2:

14 min at 94°C
followed by: 1 min at 94°C
1 min at 60°C
2 min at 72°C
for 5 cycles
followed by: 30 sec at 94°C
30 sec at 60°C
1 min at 72°C
for 25 cycles
followed by: 10 min at 72°C
followed by: 5 min at 4°C
followed by: 10°C forever

Mix for probe templates PT015-1, PT016-1, PT059-1 till PT061-1 and PT063-1:
Components for a 50 µL reaction:

- 100 pg template DNA
- 5 µL 10x Reaction Buffer with $MgCl_2$
- 1 µL 10 mM dNTPs
- 1 µL forward primer (10 pmol/µL)
- 1 µL reverse primer (10 pmol/µL)
- 0.25 µL *Taq* DNA polymerase (5U/µL)
- MQ water up to 20 µL

Thermocycling profile for probe templates PT015-1, PT016-1, PT059-1 till PT061-1 and PT063-1:

4 min at 94°C
followed by: 1 min at 94°C
1 min at 57°C
2 min at 72°C
for 5 cycles
followed by: 30 sec at 94°C
30 sec at 57°C
1 min at 72°C
for 25 cycles
followed by: 10 min at 72°C
followed by: 5 min at 4°C
followed by: 10°C forever