Title

Full DNA sequence of event insert and integration site of *Glycine max* transformation event FG72

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ABBREVIATIONS

3’	3-prime
5’	5-prime
bp	base pairs
ca.	*Circa* (Latin)
Cf.	*Confer* (Latin)
DNA	DeoxyriboNucleid Acid
Gen. DNA	Genomic DNA
i.e.	*id est* (Latin)
N.A.	Not applicable
PCR	Polymerase Chain Reaction
T-DNA	Transfer or Transgenic DeoxyriboNucleid Acid

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SUMMARY

The *Glycine max* transformation event FG72 was produced by means of direct gene transfer of soybean cells using an 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., 2009).

In this study, the FG72 inserted transgenic sequences, the 5' and 3' flanking sequences, the sequences of the newly created junctions due to the translocation, and corresponding sequences of *Glycine max* wild type regions were determined.

For the purpose of this study, genomic DNA was isolated from transgenic plants and non-transgenic plants. Seven PCR fragments were amplified to determine the sequence of the FG72 locus. Two PCR fragments were amplified to determine the sequence of the newly created junctions due to the translocation of genomic sequences, and four PCR fragments were amplified to determine sequences of *Glycine max* wild type regions. Consensus sequences were prepared and alignments of the sequences of *Glycine max* transformation event FG72 with sequences of plasmid pSF10 and with sequences of wild type *Glycine max* were performed.

The alignments show that the inserted transgenic sequences are identical to T-DNA sequences of the transforming plasmid pSF10. Twenty-four bases of the FG72 locus do not correspond to either pSF10 plasmid DNA or non-transgenic DNA and are therefore annotated as filler DNA.

The alignments demonstrate that the translocation of genomic sequences resulted in the generation of two additional junctions (fragments FG72-TL1 and FG72-TL2), of which the 3' junction is joined by 158 bp of Ph4a748 promoter sequences (fragment FG72-TL2). Twenty-five bases of the translocated sequence (fragment JACK-WT2) and two bases of the reintegration site of the translocated sequence (fragment JACK-WT3) are deleted upon transformation.

The alignments demonstrate that the non-transgenic sequences determined on the FG72 genome, *i.e.* the 5' and the 3' flanking sequences of the FG72 insert locus, the 5' and 3' end sequences of the translocated region and the sequences flanking the translocated region, are completely identical to sequences determined on the wild type locus.

1. OBJECTIVE

Bayer CropScience 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 of soybean cells.

The FG72 locus 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, resulting in the generation of two additional junctions of which the 3' junction is joined by ca. 158 bp of Ph4a748 promoter sequence (Figure 1). This translocated genomic region is depicted as region 'c' in all figures of this report.

In this study, the FG72 inserted transgenic sequences, the 5' and 3' flanking sequences, the newly created junctions due to the translocation, and the sequences of the corresponding *Glycine max* wild type regions were determined (Figure 2).

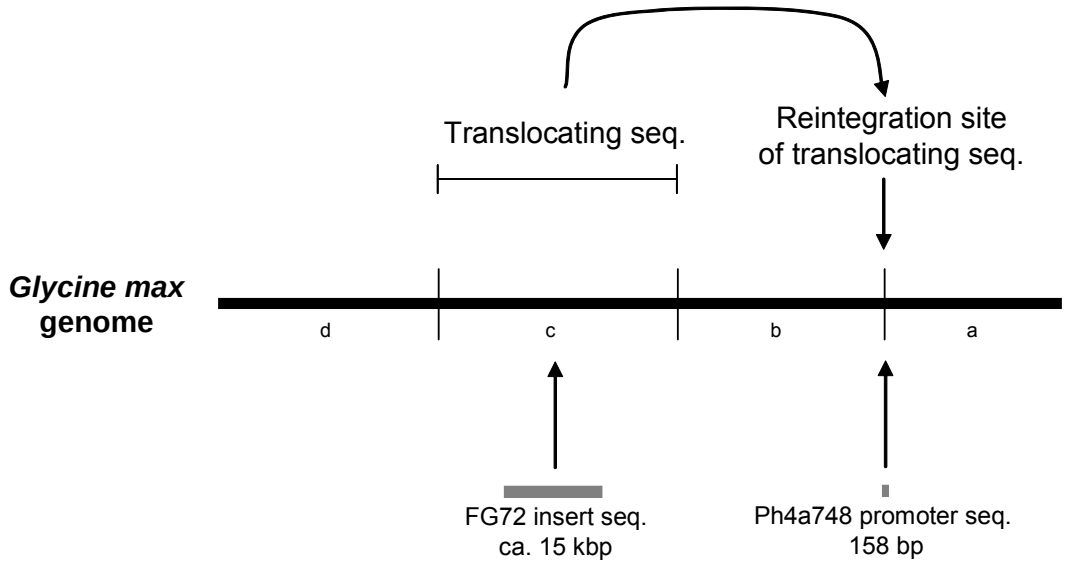

Figure 1. Integrations and translocation during transformation of *Glycine max* event FG72

2. MOLECULAR ANALYSIS

2.1. Preparation and quantification of genomic plant DNA

Seeds of *Glycine max* transformation event FG72 (seed lot 32CON0525) and seeds from the non-transgenic counterpart Jack wild type were grown in the Bayer BioScience NV greenhouse. Leaf tissues were harvested and directly frozen in liquid nitrogen.

Total genomic DNA was isolated from leaf tissue of individual plants according to Dellaporta *et al.* (1983). The DNA concentration of each preparation was determined by loading a small amount (5 µL of a 1/50 dilution) of each genomic DNA preparation on a 1% TAE agarose gel and by comparing the intensities of the obtained fragments with these of a range of λ DNA samples with known DNA concentration (25, 50, 100, 200, and 400 ng). Throughout this study, DNA preparations from multiple individual plants were used.

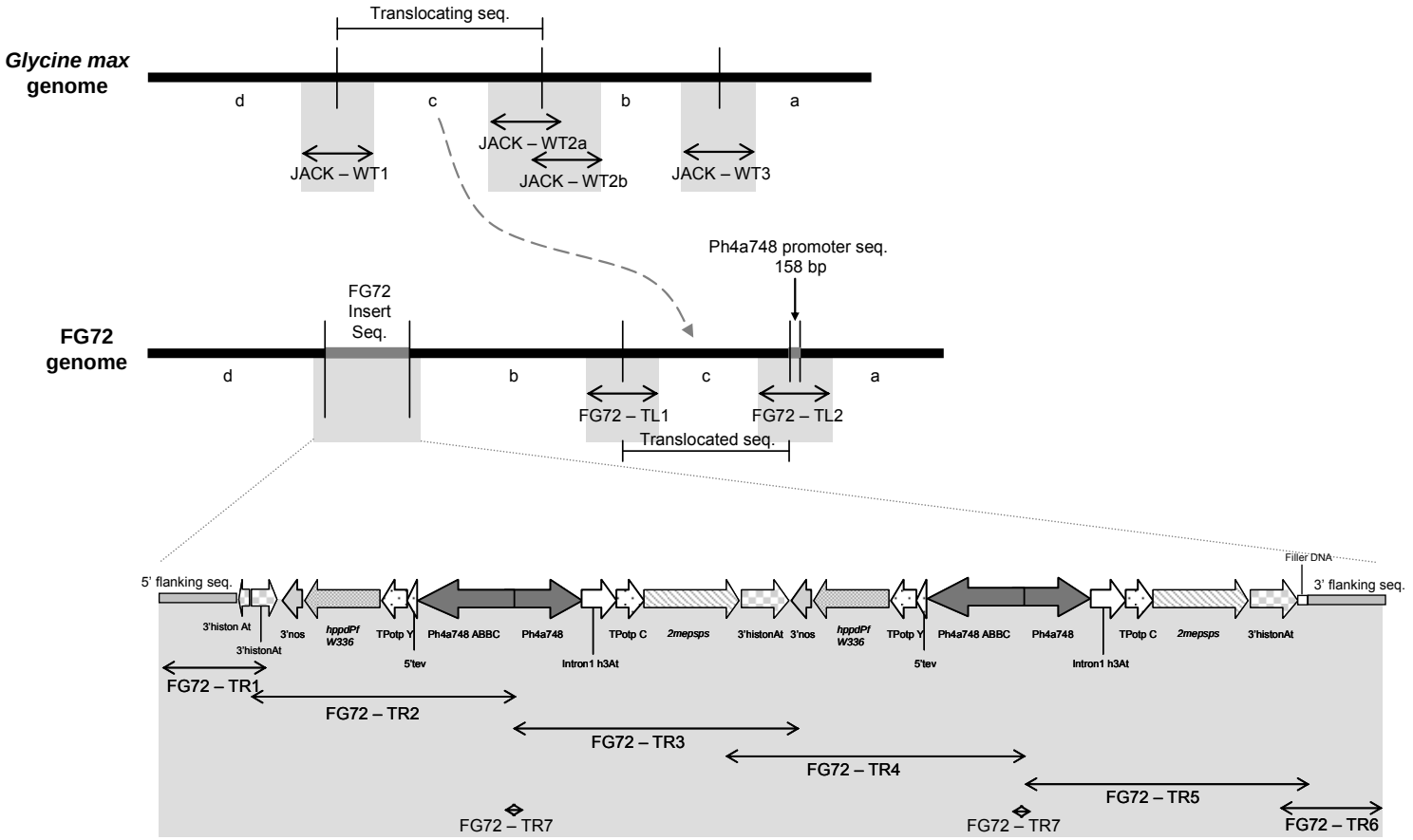

Figure 2. Schematic overview of the wild type *Glycine max* genome and the FG72 genome with indication of the fragments amplified for sequence determination (not drawn to scale)

2.2. Identity confirmation

PCRs were performed to check the identity and zygosity of the FG72 plants. Throughout this study, both hemizygous and homozygous FG72 plant material was used. The presence of the translocated region was confirmed by means of PCR.

2.3. Amplification, purification and cloning of PCR fragments

Seven PCR fragments (FG72-TR1 to FG72-TR7, Figure 2) were amplified using event specific primers and genomic DNA from *Glycine max* transformation event FG72 as template to determine the sequence of the FG72 locus. All amplified fragments are specific for one location within the FG72 locus, except for fragment FG72-TR7 which is present two times in the FG72 locus (Figure 2). The electropherograms of fragment FG72-TR7 did not show any double peaks (data not shown). Therefore it is concluded that the sequence of both copies of the FG72-TR7 fragment in the FG72 locus is exactly the same.

To determine the sequence of the newly created junctions due to the translocation of genomic sequences, two PCR fragments were amplified using genomic DNA from *Glycine max* transformation event FG72 as template (fragment FG72-TL1 and FG72-TL2, Figure 2).

To determine the sequences of the *Glycine max* wild type regions interrupted upon transformation, four fragments were amplified using genomic DNA prepared from wild type *Glycine max* (variety Jack) plants as template (Figure 2):

- fragment JACK-WT1
- fragments JACK-WT2a and JACK-WT2b
- fragment JACK-WT3

Information about the primers, template DNA and thermocycling conditions used to amplify the fragments is provided in Table 1 and Appendix 1.

In order to obtain a sufficient amount of PCR fragment, a number of identical reactions were performed as indicated in Table 1. Identical PCR reactions were pooled before purification.

The PCR fragments of interest were purified by means of the QIAquick PCR Purification kit (QIAgen) or by means of the QIAquick Gel Extraction kit (Qiagen), as indicated in Table 1.

Because the obtained amount of fragment FG72-TR2 was too low for further use, this fragment was cloned into a pCR® II-Blunt-TOPO® vector (Invitrogen) and transformed into TOP10® cells (Invitrogen). Plasmid DNA was prepared of six different colonies containing the cloned FG72-TR2 fragment using a QIAprep Spin Miniprep kit (QIAgen).

2.4. Determination of the concentration of the purified PCR fragments and vectors

To determine the DNA concentration of the purified PCR fragments, appropriate amounts of these fragments were loaded on an agarose gel, together with 2 µL, 4 µL and 8 µL of Low or High DNA Mass Ladder (Invitrogen). On completion of the electrophoresis, the size and purity of the amplified fragments was verified and the concentration of the DNA samples was estimated by comparing the intensities of the bands of the PCR fragments with the bands of the DNA Mass Ladder.

The DNA concentration of the six purified plasmids containing the cloned FG72-TR2 fragment was determined using a nanodrop device (Nanodrop technologies).

2.5. Sequence determination

All purified PCR fragments and DNA of 6 plasmids containing the FG72-TR2 fragment were sent to AGOWA GmbH (Berlin, Germany) for sequencing.

Each PCR fragment was sequenced in both directions until a 4-fold coverage was obtained. Because of the short length, fragment FG72-TR7 was sequenced 4 times in only one direction, also resulting in a 4-fold coverage.

The six plasmids containing the FG72-TR2 fragment were each sequenced in both directions until a 2-fold coverage was reached, resulting in a total 12-fold coverage for the sequence of the FG72-TR2 fragment.

The obtained electropherograms were supplied to Bayer BioScience NV.

2.6. Fragment consensus preparations and fragment assemblies

A consensus sequence of each amplified fragment was made using the Sequencher software from Gene Codes Corporation (Michigan, USA). The consensus sequence of the cloned FG72-TR2 fragment was prepared by combining the sequence results of all six plasmids containing this fragment.

For consensus sequences obtained from PCR fragments, it was verified that each base was correctly reported in at least 4 different sequence runs. For sequences obtained from the plasmids containing the FG72-TR2 fragment, it was verified that for each plasmid each base was correctly reported in at least 2 different sequence runs. Bases where the required coverage was not obtained were checked on the corresponding electropherograms and were edited using the Sequencher software. Sequences originating from the primers used for amplification of the fragment were excluded from the consensus sequences.

To assemble the ‘FG72-TR’ sequence of the complete FG72 locus, the consensus sequences of fragments FG72-TR1 through FG72-TR7 were used.

The sequence ‘JACK-WT2’ was assembled using the consensus sequences of fragments JACK-WT2a and JACK-WT2b.

An overview of all obtained sequences is given in Appendix 2 to Appendix 7.

Table 1. Details on PCR conditions and purification methods used.

Fragment ID	Template DNA	Primer pair	Primer sequence (5' → 3')	Primer position in pSF10	Master mix **	Thermo-cycling profile **	Number of identical reactions	Purification method	Length of amplicon (bp)	Overlap between fragments (bp)
FG72 - TR1	*Hin*dIII digested FG72 gen. DNA	STV162	**Critical Confidential Information removed**	/	1	A	10	PCR purification	*ca*. 1927	*ca*. 263
		MAE082		10190 → 10171						
FG72 - TR2	*Hin*dIII digested FG72 gen. DNA	STV026		/	3	B	1	Gel extraction + Cloning	*ca*. 3819	*ca*. 30
		STV065		6454 → 6436						
FG72 - TR3	FG72 gen. DNA	STV063		6425 → 6443	2	B	62	Gel extraction	*ca*. 4141	*ca*. 1049
		DPA288		3285 → 3266						
FG72 - TR4	FG72 gen. DNA	MLD108		9517 → 9536	2	B	62	Gel extraction	*ca*. 4218	*ca*. 30
		STV065		6454 → 6436						
FG72 - TR5	FG72 gen. DNA	STV063		6425 → 6443	2	B	31	Gel extraction	*ca*. 4229	*ca*. 556
		JDB003 *		/						
FG72 - TR6	FG72 gen. DNA	SHA096		10098 →10123	1	A	21	PCR purification	*ca*. 1454	N.A.
		SMP185		/						
FG72 – TR7	FG72 gen. DNA	STV286 ***		6413 → 6434	2	F	1	Gel extraction	*ca*. 81	N.A.
		TVS033		6493 → 6469						
FG72 - TL1	FG72 gen. DNA	TVS032		/	2	E	20	PCR purification	*ca*. 2257	N.A.
		TVS030		/						
FG72 - TL2	FG72 gen. DNA	TVS031		/	2	E	20	Gel extraction	*ca*. 2481	N.A.
		STV068		/						
JACK - WT1	non-transgenic "Jack" gen. DNA	TVS029		/	1	A	1	PCR purification	*ca*. 2344	N.A.
		TVS030		/						
JACK - WT2a	non-transgenic "Jack" gen. DNA	SMP188		/	1	D	31	PCR purification	*ca*. 2133	*ca*. 418
		JDB003 *		/						
JACK - WT2b	non-transgenic "Jack" gen. DNA	JDB001 *		/	1	D	31	PCR purification	*ca*. 1316	N.A.
		SMP185		/						
JACK - WT3	non-transgenic "Jack" gen. DNA	TVS031		/	4	C	1	PCR purification	*ca*. 2252	
		TVS032		/						

* Comparison of the JDB001 and JDB003 primer sequences with the obtained consensus sequence of fragment JACK-WT2 revealed that these primers are not 100% homologous to the consensus sequence.

** *Cf*. Appendix 1

*** Primer STV286 has 2 binding sites in pSF10. Only the primer binding site that leads to an amiplification product is shown.

2.7. Sequence analysis of FG72 sequences

Alignments of the sequences of *Glycine max* transformation event FG72, *i.e.* consensus sequences FG72-TR, FG72-TL1 and FG72-TL2, with the plasmid pSF10 sequence (10398 bp) and with non-transgenic *Glycine max* sequences, *i.e.* consensus sequences JACK-WT1, JACK-WT2 and JACK-WT3, were performed using the Clone Manager software.

2.7.1. FG72 transgenic locus: fragment FG72-TR

Alignment of fragment FG72-TR with the sequence of pSF10 (Figure 3) shows that:

- bases 1452 to 1650, 1651 to 2075, 2070 to 9360 and 9355 to 16614 of fragment FG72-TR are identical with respectively bases 10032 to 9834, 9948 to 10372, 3075 to 10365 and 3080 to 10339 of the pSF10 plasmid (Figure 3).
- bases 2070 to 2075 and bases 9355 to 9360 of fragment FG72-TR can be originating both from pSF10 plasmid sequence downstream of 3'histonAt and from pSF10 plasmid sequence upstream of 3' nos. These sequences have no annotation linked to them and are junction regions between (partial) T-DNA copies.
- bases 1 to 1451 and 16615 to 17806 of FG72-TR show no homology with the sequence of the transforming plasmid.

Alignment of fragment FG72-TR with non-transgenic fragment JACK-WT1 shows that bases 286 to 1451 of fragment FG72-TR are identical to bases 1 to 1166 of JACK-WT1 (Figure 3, fragment d). These bases are the 5' flanking sequence of the FG72 locus. Due to primer design, a shorter 5' flanking sequence is obtained in fragment JACK-WT1 when compared to fragment FG72-TR.

Alignment of fragment FG72-TR with non-transgenic fragment JACK-WT2 shows that bases 16639 to 17806 of fragment FG72-TR are completely identical to bases 1824 to 2991 of JACK-WT2 (Figure 3, fragment b). These bases are the 3' flanking sequence of the FG72 locus.

Bases 16615 to 16638 of the FG72 locus sequence do not correspond to the plasmid nor to the wild type genomic sequences and are therefore annotated as filler DNA (Figure 3).

In conclusion, analysis of the sequence of fragment FG72-TR (17806 bp, Appendix 2, Table 2) revealed that:

- basepairs 1 to 1451 correspond to the 5' flanking sequence,
- basepairs 1452 to 16614 correspond to T-DNA sequences,
- basepairs 16615 to 16638 correspond to filler DNA and
- basepairs 16639 to 17806 correspond to the 3' flanking sequence.

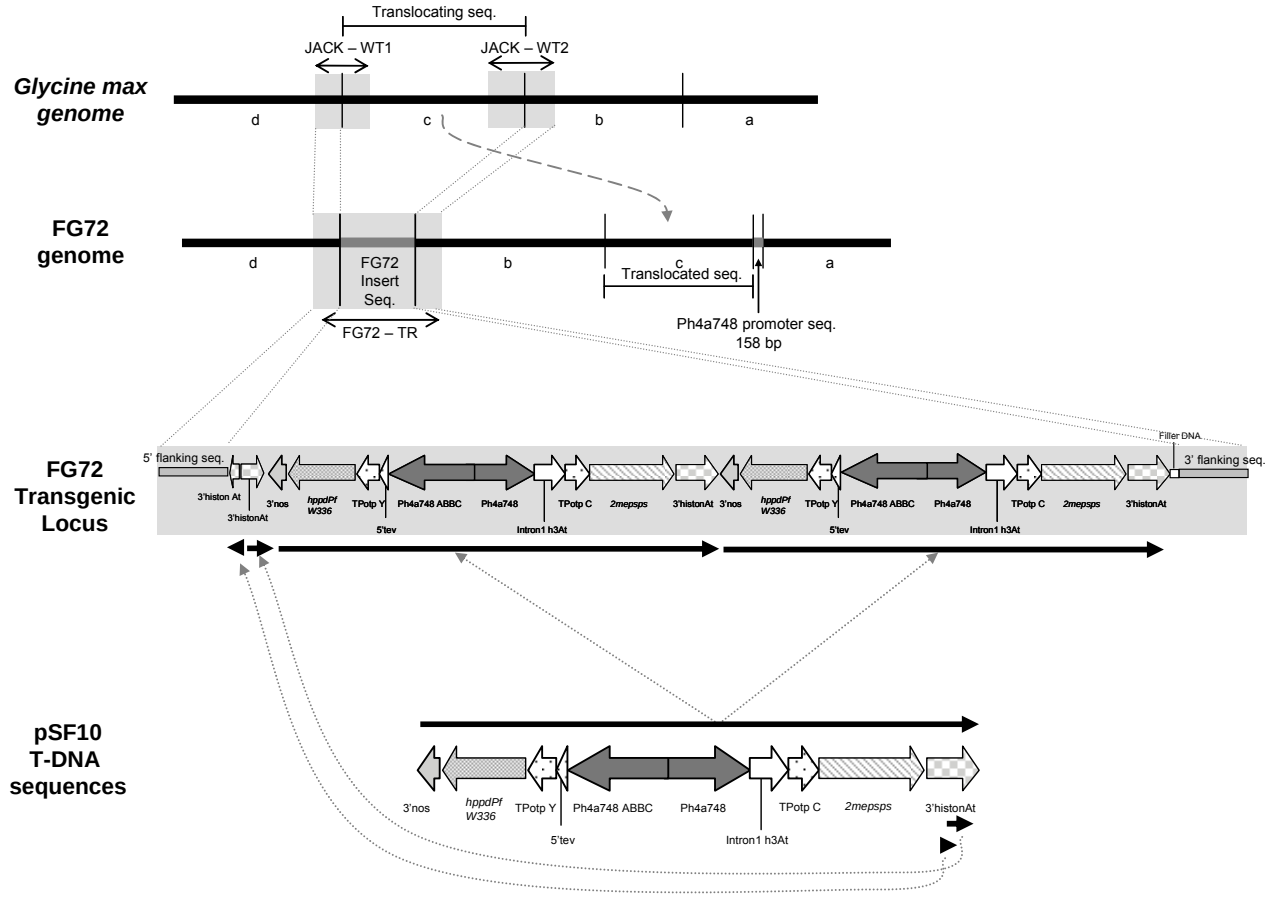

Figure 3. Annotation of fragment FG72-TR (not drawn to scale)

Sequence alignment of FG72-TR with pSF10 and non-trangenic *Glycine max* sequences:
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2.7.2. FG72 translocated region

2.7.2.1. 5' junction: fragment FG72-TL1

Alignment of fragment FG72-TL1 with the sequence of plasmid pSF10 shows no homology.

Alignment of fragment FG72-TL1 with wild type sequences (Figure 4) shows that bases 1-1082 are identical to bases 1-1082 of JACK-WT3 (Figure 4, fragment b) and that bases 1081-2217 are identical to bases 1167-2303 of JACK-WT1 (Figure 4, fragment c). Bases 1081-1082 of FG72-TL1 can be originating from fragment JACK-WT1 or JACK-WT3 and are annotated as beig originating from fragment JACK-WT1.

In conclusion, analysis of the sequence of fragment FG72-TL1 (2217 bp, Appendix 3, Table 3) revealed that:

- basepairs 1 to 1080 correspond to the 5' flanking sequence of the translocated sequence (Figure 4, fragment b),
- basepairs 1081 to 2217 correspond to 5' sequence of the translocated sequence (Figure 4, fragment c).

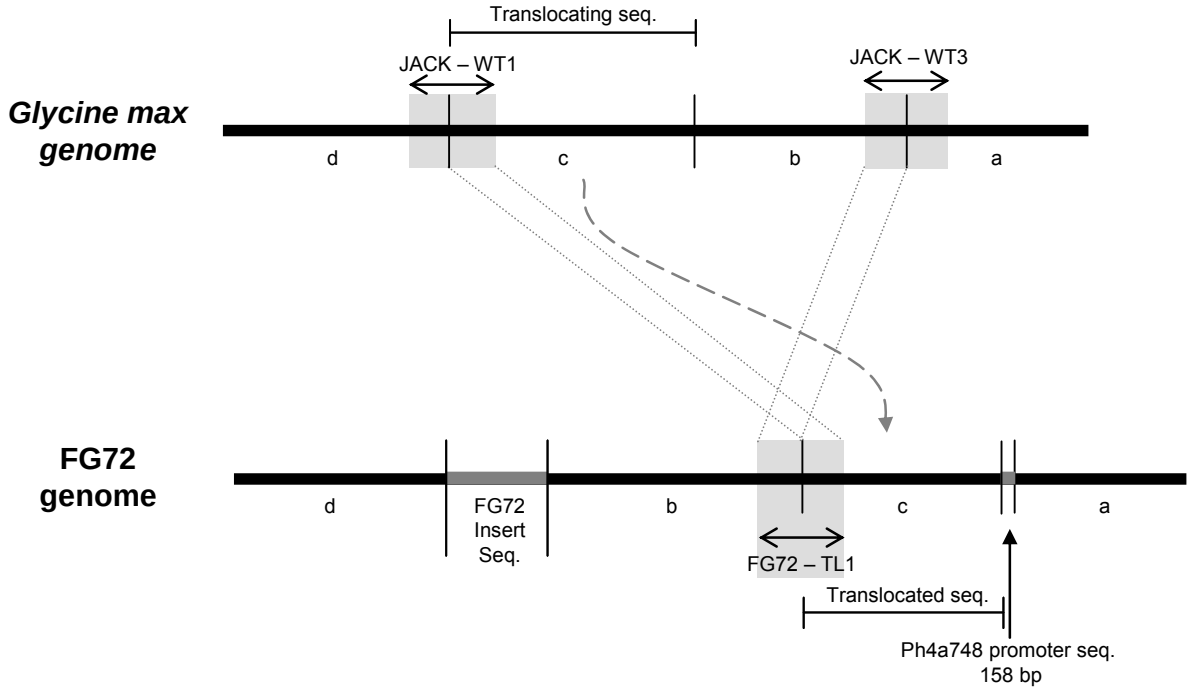

Figure 4. Annotation of fragment FG72-TL1 (not drawn to scale)

Sequence alignment of FG72-TL1 with non-trangenic *Glycine max* sequences:
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2.7.2.2. 3' junction: fragment FG72-TL2

Alignment of fragment FG72-TL2 with the sequence of vector pSF10 shows that bases 1149 to 1311 correspond to 3 identical regions in vector pSF10, *i.e.* bases 5530 to 5368 (Ph4a748 ABBC), bases 5863 to 5701 (Ph4a748 ABBC) and bases 7025 to 7187 (Ph4a748) of vector pSF10 (Figure 5). The promoters Ph4a748 and Ph4a748 ABBC are identical, except from an internal duplication in promoter Ph4a748 ABBC. The homologous region of fragment FG72-TL2 is annotated as Ph4a748 sequence.

Alignment of fragment FG72-TL2 with wild type sequences shows that bases 1-1151 are identical to bases 648-1798 of JACK-WT2 (Figure 5, fragment c). These bases correspond to the 3' sequence of the translocated region. Due to primer design, a shorter sequence is obtained in fragment FG72-TL2 when compared to fragment JACK-WT2.

Alignment of fragment FG72-TL2 with fragment JACK-WT3 shows that bases 1310-2439 of fragment FG72-TL2 are identical to bases 1083-2212 of JACK-WT3 (Figure 5, fragment a). These bases are the 3' flanking sequence of the translocation.

Bases 1149-1151 of fragment FG72-TL2 correspond to both pSF10 and JACK-WT2 sequences. They are annotated as originating from fragment JACK-WT2. Bases 1310-1311 of fragment FG72-TL2 correspond to both pSF10 and JACK-WT3 sequences and are annotated as originating from fragment JACK-WT3. As a result, only bases 1152 to 1309 of fragment FG72-TL2 are annotated as originating from Ph4a748 sequence of vector pSF10, corresponding to bases 5527 to 5370, 5860 to 5703 and 7028 to 7185 of this vector.

In conclusion, analysis of the sequence of fragment FG72-TL2 (2439 bp, Appendix 4, Table 4) revealed that:

- basepairs 1 to 1151 correspond to 3' sequence of the translocated region (Figure 5, fragment c),
- basepairs 1152 to 1309 correspond to Ph4a748 promoter sequence,
- basepairs 1310 to 2439 correspond to the 3' flanking sequence of the translocated region (Figure 5, fragment a).

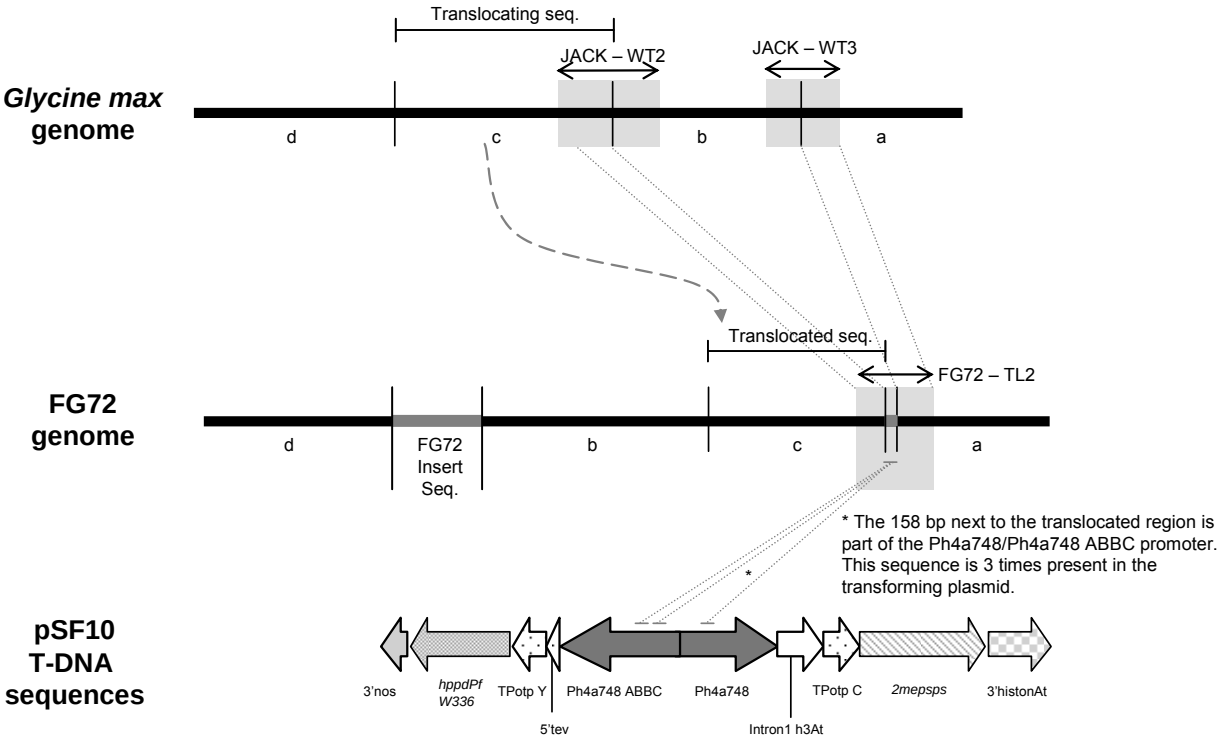

Figure 5. Annotation of fragment FG72-TL2 (not drawn to scale)

Sequence alignment of FG72-TL2 with pSF10 and non-trangenic *Glycine max* sequences:
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2.8. Sequence analysis pre insertion locus sequences

Alignments of the *Glycine max* wild type sequences, interrupted upon transformation, *i.e.* consensus sequences JACK-WT1, JACK-WT2 and JACK-WT3, with the annotated sequences of *Glycine max* event FG72, *i.e.* consensus sequences FG72-TR1, FG72-TL1 and FG72-TL2, were performed using the Clone Manager software.

2.8.1. Fragment JACK-WT1

Alignment of fragment JACK-WT1 (2303 bp,

Appendix 5, Table 5) with the annotated FG72 sequences (Figure 6) shows that:

- bases 1-1166 are completely identical to bases 286-1451 of FG72-TR, *i.e.* the 5' flanking sequence of the FG72 locus (Figure 6, fragment d),
- bases 1167-2303 are completely identical to bases 1081-2217 of FG72-TL1, *i.e.* the 5' sequence of the translocated region (Figure 6, fragment c).

Due to primer design, a shorter 5' flanking sequence is obtained in fragment JACK-WT1 when compared to fragment FG72-TR.

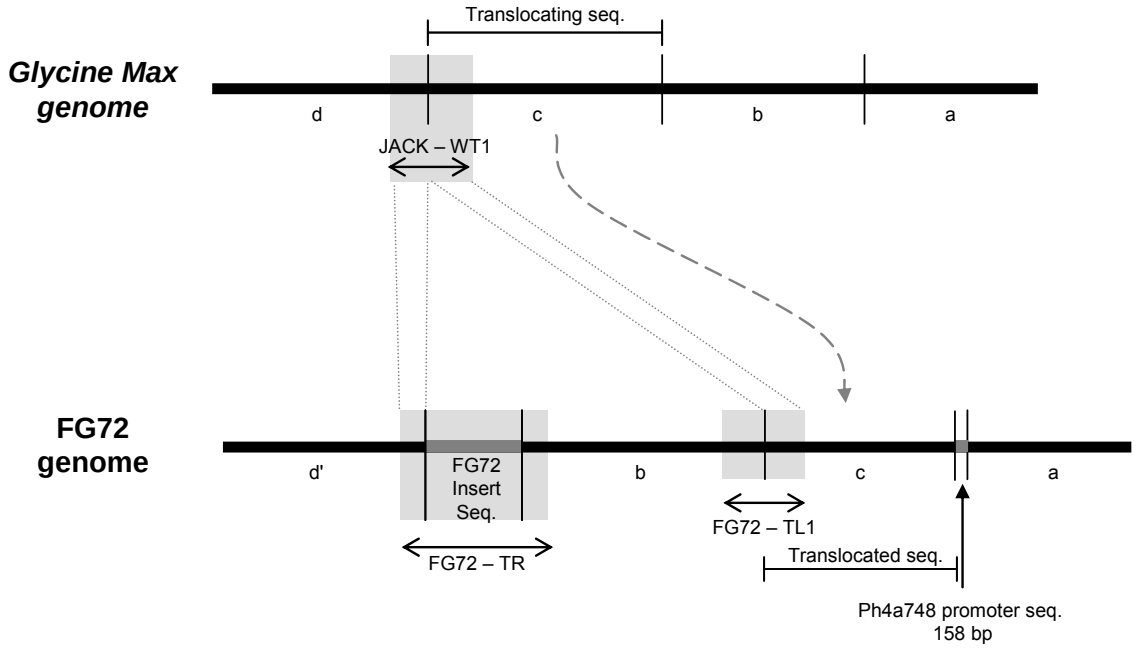

Figure 6. Annotation of fragment JACK-WT1 (not drawn to scale)

Sequence alignment of JACK-WT1 with the annotated sequences of *Glycine max* event FG72:
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2.8.2. Fragment JACK-WT2

Alignment of fragment JACK-WT2 (2991 bp, Appendix 6, Table 6) with the annotated FG72 sequences (Figure 7) shows that:

- bases 648-1798 are identical to bases 1-1151 of FG72-TL2, *i.e.* the 3' sequence of the translocated region (Figure 7, fragment c),
- bases 1824-2991 of fragment JACK-WT2 are identical to bases 16639-17806 of FG72-TR, *i.e.* the 3' flanking sequence of the FG72 locus (Figure 7, fragment b),
- bases 1799-1823 of fragment JACK-WT2 don't show homology with FG72 sequences and are annotated as bases deleted upon transformation (Figure 7).

Due to primer design, a shorter 3' translocated sequence is obtained in fragment FG72-TL2 when compared to fragment JACK-WT2.

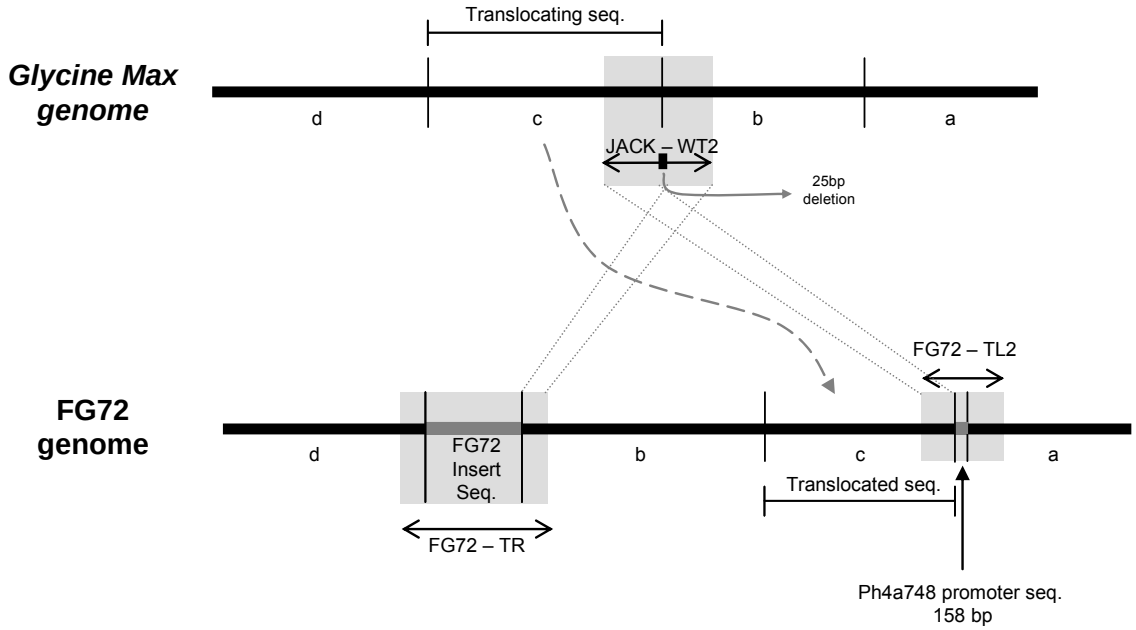

Figure 7. Annotation of fragment JACK-WT2 (not drawn to scale)

Sequence alignment of JACK-WT2 with the annotated sequences of *Glycine max* event FG72:
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2.8.3. Fragment JACK-WT3

Alignment of fragment JACK-WT3 (2212 bp, Appendix 7, Table 7) with the annotated FG72 sequences (Figure 8) shows that:

- bases 1-1082 are identical to bases 1-1082 of FG72-TL1. Since only bases 1-1080 of fragment FG72-TL1 are annotated as the 5' flanking sequence of the translocated region, also only bases 1-1080 of fragment JACK-WT3 are annotated as the 5' flanking sequence of the translocated region (Figure 8),
- bases 1083-2212 of fragment JACK-WT3 are identical to bases 1310-2439 of FG72-TL2, *i.e.* the 3' flanking sequence of the translocated region (Figure 8),
- bases 1081-1082 of fragment JACK-WT3 are bases deleted upon transformation (Figure 8).

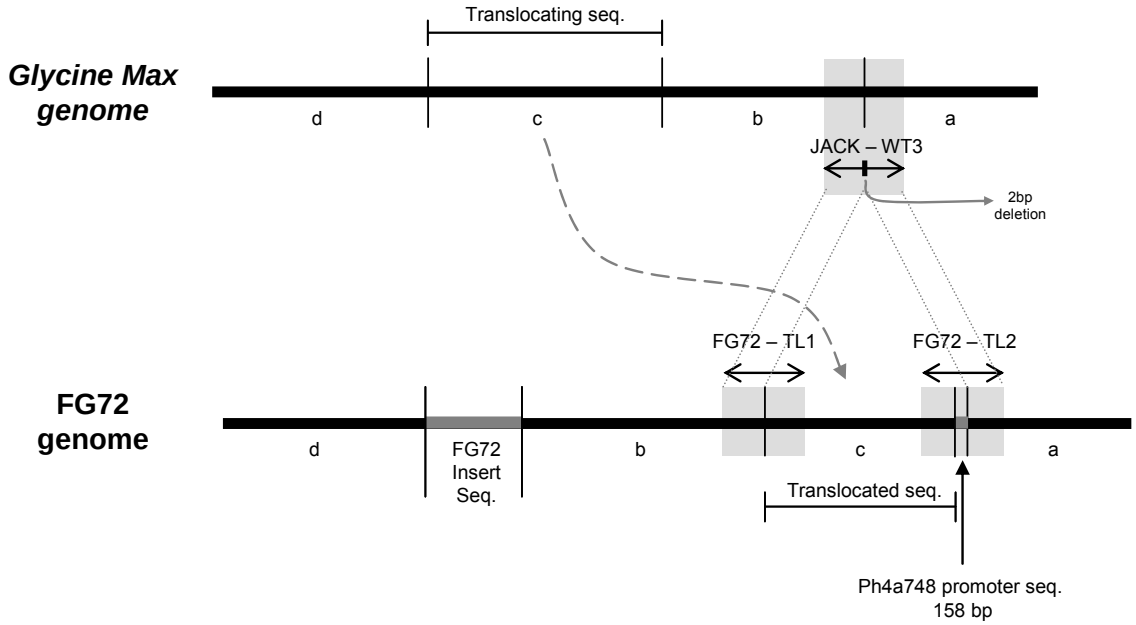

Figure 8. Annotation of fragment JACK-WT3 (not drawn to scale)

Sequence alignment of JACK-WT3 with the annotated sequences of *Glycine max* event FG72:
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3. CONCLUSION

The FG72 inserted transgenic sequences, the 5' and 3' flanking sequences, the sequences of the newly created junctions due to the translocation, and corresponding sequences of the *Glycine max* wild type regions, were determined and compared with each other.

The analyses show that the inserted transgenic sequences are identical to T-DNA sequences of the transforming plasmid pSF10. Twenty-four bases of the FG72 locus do not correspond to either pSF10 plasmid DNA or wild type genomic DNA and are filler DNA.

Upon integration of the FG72 insert into the *Glycine max* genome, a genomic region translocated to a new position. This translocation resulted in the generation of two additional junctions (fragments FG72-TL1 and FG72-TL2), of which the 3' junction is joined by 158 bp of Ph4a748 promoter sequence (fragment FG72-TL2). Twenty-five bases of the translocated sequence (fragment JACK-WT2) and 2 bases of the reintegration site of the translocated sequence (fragment JACK-WT3) are deleted upon transformation.

The analyses also demonstrate that the non-transgenic sequences determined on the FG72 genome, *i.e.* the 5' and the 3' flanking sequences of the FG72 insert locus, the 5' and 3' end sequences of the translocated region and the sequences flanking the translocated region, are completely identical to sequences determined on the wild type locus.

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2.			Verhaeghe, S. (2010) - Detailed insert characterization of *Glycine max* transformation event FG72 by means of Southern blot analysis.

Appendix 1. Thermocycling profiles and PCR reaction conditions

Thermocycling profile 'A'

Temperature	Time	# of cycles
94°C	4'	1x
94°C	1'	5x
57°C	1'	
68°C	4'	
94°C	15"	25x
60°C	45"	
68°C	4' + 5"/Cycle	
68°C	10'	1x
4°C	Forever	1x

Thermocycling profile 'B'

Temperature	Time	# of cycles
98°C	3'	1x
98°C	10"	10x
75°C -1°/Cycle	15"	
72°C	4'	
98°C	10"	35x
60°C	15"	
72°C	4'	
72°C	10'	1x
4°C	5'	1x
10°C	Forever	1x

Thermocycling profile 'C'

Temperature	Time	# of cycles
94°C	2'	1x
94°C	10"	10x
60°C	30"	
72°C	8'	
94°C	10"	35x
60°C	30"	
68°C	8' + 10"/cycle	
68°C	10'	1x
4°C	5'	1x
10°C	Forever	1x

Thermocycling profile 'D'

Temperature	Time	# of cycles
94°C	4'	1x
94°C	1'	5x
57°C	1'	
72°C	2'	
94°C	15"	25x
60°C	45"	
72°C	2'	
72°C	10'	1x
4°C	Forever	1x

Thermocycling profile 'E'

Temperature	Time	# of cycles
98°C	2'	1x
98°C	10"	30x
60°C	30"	
68°C	2'	
68°C	10'	1x
4°C	5'	1x
10°C	Forever	1x

Thermocycling profile 'F'

Temperature	Time	# of cycles
98°C	4'	1x
98°C	1'	5x
57°C	1'	
68°C	4'	
98°C	15"	25x
60°C	45"	
68°C	4' + 5"/Cycle	
68°C	10'	1x
4°C	Forever	1x

Master mix '1'

40,25 µL sterile milli-Q water
5 µL 10x Expand HF buffer with 15 mM $MgCl_2$ (Roche)
1 µL of 10 mM dNTPs
1 µL of 10 µM of each primer
0,75 µL of Expand™ High Fidelity PCR System enzyme mix (Roche)
1 µL of template DNA (~ 50 ng/µL)

Master mix '2'
20,1 µL sterile milli-Q water
6 µL 5x GC buffer (Finnzymes)
0,6 µL of 10 mM dNTPs
1 µL of 10 µM of each primer
0,3 µL of Phusion (Finnzymes)
1 µL of template DNA (~ 50 ng/µL)

Master mix '3'
20,1 µL sterile milli-Q water
6 µL 5x HF buffer (Finnzymes)
0,6 µL of 10 mM dNTPs
1 µL of 10 µM of each primer
0,3 µL of Phusion (Finnzymes)
1 µL of template DNA (~ 50 ng/µL)

Master mix '4'
39.05 µL sterile milli-Q water
5 µL 10x Expand HF buffer with 15 mM $MgCl_2$ (Roche)
1.25 µL of 10 mM dNTPs
1 µL of 10 µM of each primer
0,7 µL of Expand™ High Fidelity PCR System enzyme mix (Roche)
2 µL of template DNA (~ 50 ng/µL)

Appendix 2: Sequence of the FG72 transgenic locus, fragment FG72-TR
Critical Confidential Information removed

Table 2: Features of the FG72 transgenic locus sequences

Feature	Position	Corresponding position in pSF10
5' flanking sequence (fragment d)	bp 1 → bp 1451	/
T-DNA sequences	bp 1650 → bp 1452 bp 1651 → bp 2075 bp 2070 → bp 9360 bp 9355 → bp 16614	bp 9834 → bp 10032 bp 9948 → bp 10372 bp 3075 → bp 10365 bp 3080 → bp 10339
3'histonAt	bp 1650 → bp 1452	bp 9834 → bp 10032
3'histonAt	bp 1651 → bp 2029	bp 9948 → bp 10326
3'nos	bp 2548 → bp 2257	bp 3553 → bp 3262
hppdPf W336	bp 3625 → bp 2549	bp 4630 → bp 3554
TPotp Y	bp 3997 → bp 3626	bp 5002 → bp 4631
5'tev	bp 4138 → bp 3998	bp 5143 → bp 5003
Ph4a748ABBC	bp 5428 → bp 4139	bp 6433 → bp 5144
Ph4a748	bp 5429 → bp 6443	bp 6434 → bp 7448
Intron1 h3At	bp 6444 → bp 6924	bp 7449 → bp 7929
TPotp C	bp 6925 → bp 7296	bp 7930 → bp 8301
2mepsps	bp 7297 → bp 8634	bp 8302 → bp 9639
3'histonAt	bp 8635 → bp 9321	bp 9640 → bp 10326
3'nos	bp 9828 → bp 9537	bp 3553 → bp 3262
hppdPf W336	bp 10905 → bp 9829	bp 4630 → bp 3554
TPotp Y	bp 11277 → bp 10906	bp 5002 → bp 4631
5'tev	bp 11418 → bp 11278	bp 5143 → bp 5003
Ph4a748ABBC	bp 12708 → bp 11419	bp 6433 → bp 5144
Ph4a748	bp 12709 → bp 13723	bp 6434 → bp 7448
Intron1 h3At	bp 13724 → bp 14204	bp 7449 → bp 7929
TPotp C	bp 14205 → bp 14576	bp 7930 → bp 8301
2mepsps	bp 14577 → bp 15914	bp 8302 → bp 9639
3'histonAt	bp 15915 → bp 16601	bp 9640 → bp 10326
Filler DNA	bp 16615 → bp 16638	/
3' flanking sequence (fragment b)	bp 16639 → bp 17806	/

Appendix 3: Sequence of fragment FG72-TL1
Critical Confidential Information removed

Table 3: Features of fragment FG72-TL1

Position	Feature
bp 1 → bp 1080	5' flanking sequence of the translocated region (fragment b)
bp 1081 → bp 2217	5' sequence of the translocated region (fragment c)

Appendix 4: Sequence of fragment FG72-TL2
Critical Confidential Information removed

Table 4: Features of fragment FG72-TL2

Position	Feature
bp 1 → bp 1151	3' sequence of the translocated region (fragment c)
bp 1152 → bp 1309	Ph4a748 sequence
bp 1310 → bp 2439	3' flanking sequence of the translocated region (fragment a)

Appendix 5: Sequence of fragment JACK-WT1
Critical Confidential Information removed

Table 5: Features of fragment JACK-WT1

Position in the pre-insertion locus	Feature
bp 1 → bp 1166	5' flanking sequence of FG72 (fragment d)
bp 1167 → bp 2303	5' translocated sequence (fragment c)

Appendix 6: Sequence of fragment JACK-WT2
Critical Confidential Information removed

Table 6: Features of fragment JACK-WT2

Position	Feature
bp 1 → bp 1798	3' translocated sequence (fragment c)
bp 1799 → bp 1823	Bases deleted upon transformation
bp 1824 → bp 2991	3' flanking sequence of FG72 (fragment b)

Appendix 7: Sequence of fragment JACK-WT3
Critical Confidential Information removed

Table 7: Features of fragment JACK-WT3

Position	Feature
bp 1 → bp 1080	5' flanking sequence of the translocated region (fragment b)
bp 1081 → bp 1082	Bases deleted upon transformation
bp 1083 → bp 2212	3' flanking sequence of the translocated region (fragment a)