A Complete Sequence of the pGA1611 Binary Vector

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We report the nucleotide sequence of the binary vector pGA1611, which is used for the transformation of foreign DNA into rice. This vector is 13,476 bp long. The 5577-bp T-DNA region consists of a 1987-bp ubiquitine promoter region, 45 bp for the multiple cloning site, a 253-bp *nos* terminator region, and the 2045-bp *CaMV35S-hph-T7* chimaeric gene. The vector backbone (7004 bp) carries *oriT, traJ, trfA, tetA, tetR,* and *oriV.* An 892-bp RB region and the 489-bp LB region are also present. The T-DNA possesses 15 unique sites, six of which are at the multiple cloning site. This information will be valuable for cloning foreign DNA and modifying the vector.

Keywords: Binary vector, pGA1611, rice, sequence, transformation

Binary vectors, based on the Ti plasmid of *Agrobacterium*, have been widely used for transforming a variety of monocot and dicot plant species (Bytebier et al., 1987; Hiei et al., 1994; Ishida et al., 1996; Birch, 1997; Binns, 2002). These vectors have been modified to achieve various objectives, such as the expression of a foreign gene in a new host plant (Song et al., 2003) and antisense/RNAi suppression for functional analysis of an endogenous gene (Pnueli et al., 1994). They have also been used for tagging genes through random insertional mutagenesis, and for trapping genes via gene fusion (Koncz et al., 1992; Jeon and An, 2001; Jung et al., 2003). Finally, binary vectors have been proven efficient in studies of expression by employing a reporter gene such as *gus* (Jeon et al., 1999).

Despite their usefulness, these vectors are not easy to manipulate and utilize because they are usually larger than 10 kb, and contain many enzyme sites. In addition, the vector backbone sequence is frequently integrated into chromosomes along with the T-DNA in both dicots (de Buck et al., 2000) and monocots (Kim et al., 2003). This integrated sequence influences transgene expression (Iglesias et al., 1997; Matzke and Matzke, 1998; Jakowitsch et al., 1999). When plants are commercialized, they must not contain those sequences because the vector backbones are derived from bacteria and may cause undesirable characteristics. Therefore, knowing the entire sequence of a binary vector facilitates the cloning of a foreign gene and manipulation of the vector. Moreover, that information becomes valuable for analyzing transgenic plants.

We had previously developed a binary vector, pGA1611, that could be used for transforming a foreign DNA fragment into rice (Lee et al., 1999). That vector had been modified to fill a variety of roles, including the generation of T-DNA tagging vectors pGA2144, pGA2707, and pGA2715. These have now been used for producing T-DNA taggings and activation taggings of rice genes (Jeon et al., 2000; Jeong et al., 2002).

We have previously described the construction of pGA1611 (Lee et al., 1999). Its T-DNA region harbors the maize ubiquitin (*ubi*) promoter and the *nopaline synthase* (*nos*) terminator near the right border (RB).

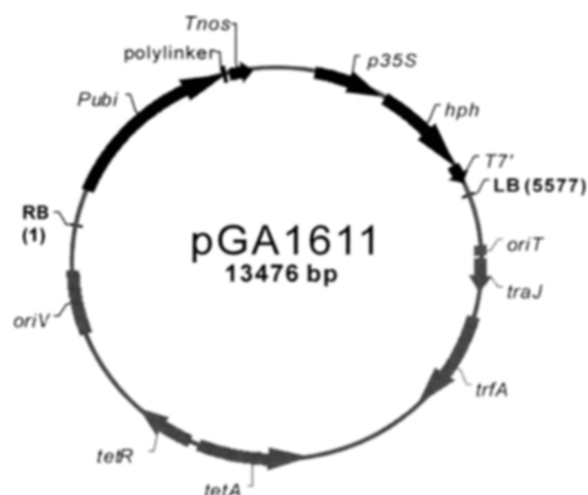

Figure 1. Map of the pGA1611 binary vector. Components are described in Table 1.

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For cloning foreign DNA, the unique HindIII, SacI, HpaI, and KpnI sites are located between those two components. When a foreign gene is inserted in the right orientation, it can be ectopically expressed in plant cells. As a selectable marker for plant transformation, a fusion gene complex (Fig. 1) that comprises the *CaMV 35S* promoter, the *hygromycin phosphotransferase* (*hph*) coding region, and the termination region of the T-DNA gene *7* is located near the left border (LB). Furthermore, the vector backbone sequence on the binary vector is derived from pGA472 (An et al., 1985). The backbone carries the origin of replication (*oriV*) as well as the selectable marker, tetracycline resistance operon (*tetA* and *tetR*) (Table 1 and Fig. 1). It also contains regions *oriT*, *traJ*, and *trfA* for transferring the plasmid from one bacterium to another.

We have sequenced the pGA1611 vector using the ABI PRISM® 3100 Genetic Analyzer System and synthetic origomers, which are designed based on the known regions of the vector (GenBank accession no. AY373338). The vector is 13,476 bp long. Its T-DNA region is 5577 bp and consists of the ubiquitine pro-

Table 1. Components of the pGA1611 binary vector

Component	Position	Description	Litcrature Cited
RB	1..25	RB of Ti-plasmid	An et al. (1985)
pUbi	420..2406	promoter of the maize *ubiquitin* gene	Christensen et al. (1992); Toki et al. (1992)
Polylinker	2407..2451	multiple cloning site	
Tnos	2463..2715	3'UTR of the *nos* gene	Bevan et al. (1983); Toki et al. (1992)
p35S	3366..4108	35S promoter of CaMV	Franck et al. (1980)
hph	4151..5176	hygromycin phosphotransferase gene	Kaster et al. (1983)
T7'	5199..5410	terminator sequence of T-DNA gene *7*	Gielen et al. (1984)
LB	5553..5577	LB of Ti-plasmid	An et al. (1985)
oriT	6132..6241	*incP* origin of transfer	An et al. (1985)
traJ	6274..6645	*oriT*-recognizing protein	An et al. (1985)
trfA	6917..8065	transacting replication	An et al. (1985)
tetA	9338..10537	tetracycline resistance gene	An et al. (1985)
tetR	10643..11293	tetracycline resistance regulation	An et al. (1985)
oriV	12288..12996	*incP* origin of replication	An et al. (1985)

Table 2. Restriction enzymes that cut pGA1611 once, sorted sequentially by base-pair number.

Enzyme	Cut site	Recognition sequence
PmeI	39	gttt/aaac
AocI, Bse21I, Bsu36I, CvnI, Eco81I	247	cc/tnagg
BamHI, BstI	408	g/gatcc
PaeR7I, Sfr274I, XhoI	1115	c/tcgag
BglII	1362	a/gatct
HindIII	2425	a/agctt
Ecl136II, EcoICRI	2436	gag/ctc
Psp124BI, SacI, SstI	2438	gagct/c
HpaI	2442	gtt/aac
Acc65I, Asp718I	2446	g/gtacc
KpnI	2450	ggtac/c
AflII, BfrI, BspTI, Bst98I, MspCI, Vha464I	2492	c/ttaag
BstEII, BstPI, Eco91I, EcoO65I, PspEI	2768	g/gtnacc
BsaI, Eco31I	3202	ggtctc
SgfI	4515	gcgat/cgc
AhdI, AspEI, Eam1105I, EclHKI	5884	gacnnn/nngtc
SfiI	7271	ggccnnnn/nggcc
AvrII, BlnI	9271	c/ctagg
AatI, Eco147I, Pme55I, SseBI, StuI	9321	agg/cct
BstXI	9560	ccannnnn/ntgg
EcoNI	11003	cctnn/nnnagg
AgeI, PinAI	11706	a/ccggt
BstSNI, Eco105I, SnaBI	11998	tac/gta
CciNI, NotI	12885	gc/ggccgc
BbuI, PaeI, SphI	13394	gcatg/c

moter (420-2406) followed by multiple cloning sites (2407-2451) and the *nos* terminator (2463-2715). The chimaeric gene fusion of *CaMV35S-hph-T7* is present at 3366-5410. Our vector backbone sequence is derived from a wide host-range plasmid, pTJS75 (An et al., 1985). This 7004-bp backbone carries *oriT* (6132-6241), *traJ* (6274-6528), *trfA* (6917-8065), *tetA* (9338-10537), *tetR* (10643-11293), and *oriV* (12288-12996). The 892-bp RB region (12997-412) and the 489-bp LB region (5504-5992) have been derived from the nopaline-type Ti plasmid pTiT37.

Those restriction enzymes that cut pGA1611 once are described in Table 2. The T-DNA possesses 15 unique sites, with 6 being found at the multiple cloning site. Within the vector backbone, 10 unique sites are present. This information will be valuable in cloning foreign DNA and modifying the vector.

ACKNOWLEDGEMENT

This work was supported, in part, by grants from the Crop Functional Genomic Center (CG-1111), the 21 Century Frontier Program, from the Biogreen 21 Program, Rural Development Administration, and from POSCO. The study was also funded by the National Research Laboratory Program of the Korea Institute of Science and Technology Evaluation and Planning.

Received June 5, 2003; accepted August 30, 2003.

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