pTN290 and pPZP201-PMI-sGFP construction; pPZP201, pPZP212 sequences; (edited 7/12/2010)

From: Fu et al. 2004
pPZP201-PMI-sGFP Plasmid construction and plant transformation (derived from pPZP201)
The *sgfp* expression matrix (*Ubi*-1::*sgfp*::*nos*3′) was constructed from the *sgfp* (S65T) gene (Chiu et al. 1996) and was initially loaded into the T-DNA region of the binary vector **pPZP201** (Hajdulkiewicz et al. 1994) that generated the plasmid pUGFP (*Ubi*-1::*sgfp*). To create the dual marker *manA-gfp*, the *E. coli manA* expression cassette (*Ubi*-1::*manA*::35S3′) on the plasmid vector pRT104 was released by the *Hind*III restriction enzyme and was subcloned upstream of the *sgfp* expression matrix in the plasmid, pUGFP. The orientation of the insert was confirmed by digestion of the recombinant plasmid and gel analysis. The derived pPMI–GFP plasmid (Fig. 1) was mobilized into the *A. tumefaciens* strain, EHA 101 (Hood et al. 1986), by electroporation (Mozo and Hooykaas 1991).

From: Howe et al. 2006
pPTN290-nptII-GUSplus plasmid construction (derived from pPZP212, Hajdukiewicz et al. 1994)
The *Agrobacterium tumefaciens* strain NTL4 (Luo et al. 2001) harboring the disarmed Chry5 Ti plasmid (Palanichelvam et al. 2000) designated pTiKPSF2, (NTL4/Chry5), containing the plasmid pPTN290 (Fig. 1) was streaked on LB medium containing 100 mg l–1 erythromycin, spectinomycin, and streptomycin from frozen glycerol stocks and grown for 2 days at 28◦C. **The vector pPTN290 is a derivative of pPZP212** (Hajdukiewicz et al. 1994) that carries a GUS*Plus*TM cassette under the control of the maize ubiquitin 1 promoter coupled with its first intron. The GUS*Plus*TM (CAMBIA) open reading frame (ORF) was engineered by PCR to introduce an *Nco* I and *Xba* I site at the 5_ and 3_ end, respectively, using the plasmid pCAMBIA1301 (CAMBIA) as template DNA in the reaction. The derived PCR product was subsequently fused to the tobacco etch translational enhancer element (TEV) (Carrington and Freed 1990) and subcloned between the maize ubiquitin 1 promoter and the 3_ UTR of the CaMV 35S transcript. The GUS*Plus*TM ORF in pPTN290 is interrupted with the rice catalase intron (CAMBIA) to prevent expression in the bacterium.
Plant Cell, Tissue and Organ Culture **75:** 1–18, 2003. S003 *Kluwer Academic Publishers. Printed in the Netherlands.*

Review of Plant Biotechnology and Applied Genetics
Optimisation of transformation conditions and production of transgenic sorghum (*Sorghum bicolor*) via microparticle bombardment Yohannes Tadesse1, La´szlo´ Sa´gi2,*, Rony Swennen2 & Michel Jacobs

Email message from L. Sagi to TM on 7/19/10
A forward primer 5'-GAGGCTATTCGGCTATGACTG-3' and a reverse primer 5'-ATCGGGAGCGGCGATACCGTA -3' can be used to amplify a 700-bp portion of the neo gene.

nuccore

gi|506657|gb|U10462.1|CVU10462 Binary cloning vector pPZP212 for plant transformation, complete sequence
nptII nptIIF nptIIR NcoI SphI ClaI PvuII

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Neomycin phosphotransferase II (kanamycin resistance gene)-sequence from Hajdukiewiz 1998 (pPZP111)

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pPZP201 complete sequence

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