**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.



gi|506657|gb|U10462.1|CVU10462 Binary cloning vector pPZP212 for plant transformation, complete sequence

nptII nptIIF nptIIR NcoI SphI ClaI PvuII

AGTACTTTGATCCAACCCCTCCGCTGCTATAGTGCAGTCGGCTTCTGACGTTCAGTGCAGCCGTCTTCTG

AAAACGACATGTCGCACAAGTCCTAAGTTACGCGACAGGCTGCCGCCCTGCCCTTTTCCTGGCGTTTTCT

TGTCGCGTGTTTTAGTCGCATAAAGTAGAATACTTGCGACTAGAACCGGAGACATTACGCCATGAACAAG

AGCGCCGCCGCTGGCCTGCTGGGCTATGCCCGCGTCAGCACCGACGACCAGGACTTGACCAACCAACGGG

CCGAACTGCACGCGGCCGGCTGCACCAAGCTGTTTTCCGAGAAGATCACCGGCACCAGGCGCGACCGCCC

GGAGCTGGCCAGGATGCTTGACCACCTACGCCCTGGCGACGTTGTGACAGTGACCAGGCTAGACCGCCTG

GCCCGCAGCACCCGCGACCTACTGGACATTGCCGAGCGCATCCAGGAGGCCGGCGCGGGCCTGCGTAGCC

TGGCAGAGCCGTGGGCCGACACCACCACGCCGGCCGGCCGCATGGTGTTGACCGTGTTCGCCGGCATTGC

CGAGTTCGAGCGTTCCCTAATCATCGACCGCACCCGGAGCGGGCGCGAGGCCGCCAAGGCCCGAGGCGTG

AAGTTTGGCCCCCGCCCTACCCTCACCCCGGCACAGATCGCGCACGCCCGCGAGCTGATCGACCAGGAAG

GCCGCACCGTGAAAGAGGCGGCTGCACTGCTTGGCGTGCATCGCTCGACCCTGTACCGCGCACTTGAGCG

CAGCGAGGAAGTGACGCCCACCGAGGCCAGGCGGCGCGGTGCCTTCCGTGAGGACGCATTGACCGAGGCC

GACGCCCTGGCGGCCGCCGAGAATGAACGCCAAGAGGAACAAGCATGAAACCGCACCAGGACGGCCAGGA

CGAACCGTTTTTCATTACCGAAGAGATCGAGGCGGAGATGATCGCGGCCGGGTACGTGTTCGAGCCGCCC

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CGGCCAGCTTGGCCGCTGAAGAAACCGAGCGCCGCCGTCTAAAAAGGTGATGTGTATTTGAGTAAAACAG

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AGCTTGAGCTTGGATCAGATTGTCGTTTCCCGCCTTCAGTTTAAACTATCAGTGTTTGACAGGATATATT

GGCGGGTAAACCTAAGAGAAAAGAGCGTTTATTAGAATAACGGATATTTAAAAGGGCGTGAAAAGGTTTA

TCCGTTCGTCCATTTGTATGTGCATGCCAACCACAGGGTTCCCCTCGGGATCAA

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

tcagaaga actcgtcaag

6661 aaggcgatag aaggcgatgc gctgcgaatc gggagcggcg ataccgtaaa gcacgaggaa

6721 gcggtcagcc cattcgccgc caagctcttc agcaatatca cgggtagcca acgctatgtc

6781 ctgatagcgg tccgccacac ccagccggcc acagtcgatg aatccagaaa agcggccatt

6841 ttccaccatg atattcggca agcaggcatc gccatgtgtc acgacgagat cctcgccgtc

6901 gggcatgcgc gccttgagcc tggcgaacag ttcggctggc gcgagcccct gatgctcttc

6961 gtccagatca tcctgatcga caagaccggc ttccatccga gtacgtgctc gctcgatgcg

7021 atgtttcgct tggtggtcga atgggcaggt agccggatca agcgtatgca gccgccgcat

7081 tgcatcagcc atgatggata ctttctcggc aggagcaagg tgagatgaca ggagatcctg

7141 ccccggcact tcgcccaata gcagccagtc ccttcccgct tcagtgacaa cgtcgagcac

7201 agctgcgcaa ggaacgcccg tcgtggccag ccacgatagc cgcgctgcct cgtcctggag

7261 ttcattcagg gcaccggaca ggtcggtctt gacaaaaaga accgggcgcc cctgcgctga

7321 cagccggaac acggcggcat cagagcagcc gattgtctgt tgtgcccagt catagccgaa

7381 tagcctctcc acccaagcgg ccggagaacc tgcgtgcaat ccatcttgtt caatccccat

7441 g

pPZP201 complete sequence

1 agtactttga tccaacccct ccgctgctat agtgcagtcg gcttctgacg ttcagtgcag

61 ccgtcttctg aaaacgacat gtcgcacaag tcctaagtta cgcgacaggc tgccgccctg

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181 tagaaccgga gacattacgc catgaacaag agcgccgccg ctggcctgct gggctatgcc

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481 ctgcgtagcc tggcagagcc gtgggccgac accaccacgc cggccggccg catggtgttg

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901 acggccagga cgaaccgttt ttcattaccg aagagatcga ggcggagatg atcgcggccg

961 ggtacgtgtt cgagccgccc gcgcacgtct caaccgtgcg gctgcatgaa atcctggccg

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1261 cctgcaactc gccggggccg atgttctgtt agtcgattcc gatccccagg gcagtgcccg

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2581 tgatccgcta cgagcttcca gacgggcacg tagaggtttc cgcagggccg gccggcatgg

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3001 tcacagaagg caagaacccg gacgtgctga cggttcaccc cgattacttt ttgatcgatc

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3361 aaggtcgaaa aggtctcttt cctgtggata gcacgtacat tgggaaccca aagccgtaca

3421 ttgggaaccg gaacccgtac attgggaacc caaagccgta cattgggaac cggtcacaca

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3661 gtcggcctat cgcggccgct ggccgctcaa aaatggctgg cctacggcca ggcaatctac

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