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Short communication

E. coli chromosomal DNA in a transgene locus created by microprojectile bombardment in tobacco

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In our studies of the factors that regulate the expression of foreign genes (transgenes) introduced into plant genomes, we routinely use microprojectile bombardment (Allen et al., 2000). This approach has found widespread application in agronomically important plant species (such as wheat, maize and barley) where most genotypes are still recalcitrant to transformation by *Agrobacterium tumefaciens*. In the process of characterising the integration site of a transgene in the tobacco genome, we have discovered a 260 base pair fragment of *E. coli* chromosomal DNA. This DNA was found at the transgene locus despite the fact that standard procedures were implemented to remove contaminating *E. coli* chromosomal DNA from the plasmid DNA used in the transformation. It is important to stress that we have documented a single case. We do not know how common contaminating *E. coli* chromosomal DNA might be at transgene loci. Detection of such sequences is difficult without sequencing, and sequencing of transgene loci is rarely carried out. We can say that the particular *E. coli* fragment we found in one transgenic line is not found in any of 10 additional transgenic lines that were generated using the same plasmid preparations. Thus, incorporation of this particular *E. coli* fragment appears to be a rare event and is not due to incorporation of the fragment into the plasmid used for transformation.

Although we know of no other documentation of contaminating *E. coli* chromosomal DNA at transgene loci, plasmid backbone DNA is often found there. Supercoiled bacterial plasmids containing transgene inserts are most commonly used for microprojectile bombardment. Thus, the plasmid backbone is found at essentially all transgene integration sites (Pawlowski & Somers, 1998). This can be prevented by using purified linear fragments of the transgene insert for the transformation (Fu et al., 2000). In *Agrobacterium*-mediated transformation, it is generally assumed that only T-DNA between the right and left border sequences is incorporated into the host genome. Backbone DNA of the Ti plasmid, however, may accompany the T-DNA more frequently than is generally appreciated (Smith, 1998).

One might ask if plasmid backbone DNA or contaminating bacterial chromosomal DNA at the transgene integration site does any general harm to the host or is in any way detrimental to the expression of the transgene. Iglesias et al. (1997) observed silencing of transgenes that had integrated with backbone binary vector sequences but not of transgenes with T-DNA only. Bacterial DNA *per se* does not appear to pose threats to eukaryotic genomes. Indeed, bacteriophage DNA ingested by mice has been shown to become stably incorporated into the genome of somatic mouse cells without deleterious effects (Schubbert et al., 1997), and over 200 sequences of apparent bacterial origin have been found in the human genome (Lander et al., 2001). Nevertheless, the purity of DNA used for transformation and the prevention of unwanted DNA in host genome is a matter to be considered. This is especially true if gene therapy is involved or if transgenic organisms become involved in the human food chain (Levy et al., 2000). Sensitive methods have been developed to detect bacterial chromosomal DNA in plasmid preparations (Smith et al., 1999) and to eliminate the contaminating DNA (Schluep & Cooney, 1998). Such approaches should

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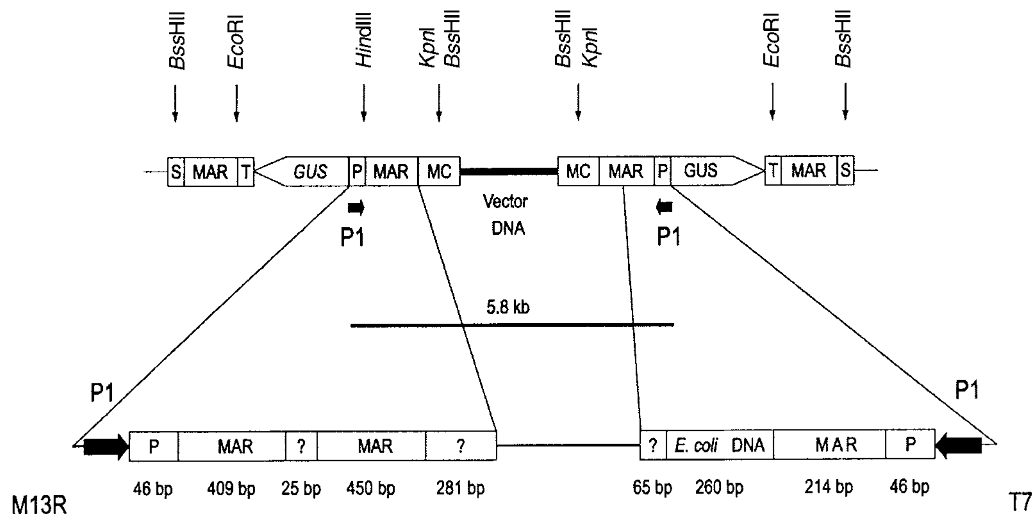

Figure 1. Sequencing results of a head to head repeat of the *GUS* gene in transgenic line DH-106. The upper portion of the figure is a model of a head to head repeat of two *GUS* copies that could be detected by PCR using the P1 primer alone. The lower portion represents actual sequences found in the cloned PCR fragment generated by the P1 primer. MAR = tobacco RB7 matrix attachment region. P = CaMV 35S promoter. T = *nos* terminator. MC/S = multiple cloning site in pBluescript II SK. '?' = sequence with no match in the GenBank data base, presumed to be plant DNA. M13R, T7 = sequencing primers. The 260 bp *E. coli* fragment is part of the *E. coli livF* gene coding region.

probably be used routinely to diminish the chances of incorporation of bacterial chromosomal DNA into the genomes of transgenic eukaryotic organisms.

We have characterised the complex transgene loci of several plants that we previously obtained by microprojectile bombardment (Ülker et al., 1999). These plants were transformed by 'co-bombardment' in which the selectable marker (neomycin phosphotransferase-*NPTII*) and the reporter gene cassette (β-glucuronidase-*GUS*) were introduced on separate plasmids. The plasmids containing these sequences were purified using a commercially available kit, and no signs of contaminating *E. coli* chromosomal DNA were visible on heavily overloaded ethidium bromide stained gels of the purified plasmids. One transgenic plant line (DH-106) is a doubled haploid that contains 15 complete copies of the *GUS* gene per haploid genome. Two of the copies are arranged as head to head repeats (Figure 1). The DNA between the *GUS* repeats was amplified by PCR and the 5.8 kb fragment was sequenced. In addition to the expected DNA, we found fragments whose sequences match nothing in the GenBank database (presumably tobacco sequences) and a 260 bp fragment which is an exact match to a sequence in the coding region of the *E. coli livF* gene (Accession J05516). This gene is part of the *E. coli* leucine-specific transport (LS-BP; LIV-BP) system (*livHMGF* genes). We used PCR to verify that this *E. coli* DNA is in the transformed plant (Figure 2). Primers

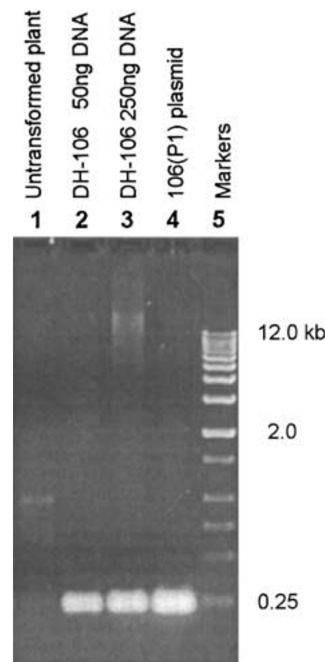

Figure 2. PCR analysis using primers located in the *E. coli* DNA. PCR with the primers located in the 260 bp *E. coli* DNA was used to confirm the presence of *E. coli* DNA in transgenic line DH-106. Templates used were: lane 1, 50 ng DNA from an untransformed tobacco plant (*Nicotiana tabacum* cv Petite Havana SR1); lanes 2 and 3, 50 ng and 250 ng of DNA from DH-106; lane 4, 1 ng of plasmid 106(P1) which contained the 5.8 kb fragment amplified with primer P1 from line DH-106. Lane 5, molecular weight markers.

located within the *E. coli* fragment amplify the expected 230 bp fragment from DNA of the transformed plant line (lanes 2 and 3) but not from DNA of untransformed plants (lane 1) or from DNA of any of 10 additional plant lines that were transformed with the same plasmid preparations (data not shown).

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