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Efficient transformation of *Agrobacterium* spp. by high voltage electroporation

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Transformation of agrobacteria has hitherto been limited to a few isolates, and at best gives only 10^3 transformants/µg (1). Plasmids are therefore usually introduced by conjugative transfer from *Escherichia coli*. We have found that efficient transformation of two strains widely used in plant genetic manipulation can be obtained by the application of a high voltage electric pulse under conditions similar to those giving high frequency transformation of *E. coli* (2). Since only small amounts of plasmid DNA are required, electroporation also opens up the possibility of direct cloning in *Agrobacterium* without the need to use *E. coli* as an intermediate host.

Strains: *A. tumefaciens* LBA4404 (pRAL4404), a derivative of Ach5; *A. rhizogenes* LBA9402 (pRi1855), a rif^r derivative of NCPPB 1855.

Preparation of cells: Agrobacterial cultures were grown at 29° for 24-30 h in 2 X YT medium to an OD_{600} of 0.5-0.7. Cells were cooled on ice, pelleted, washed successively in 1, 0.5, 0.02 and 0.02 culture volumes of cold 10% (v/v) glycerol and resuspended in 0.01 volume 10% glycerol (10^{11}-10^{12} cells/ml). Aliquots were frozen in liquid N_2 and could be stored at -70° for at least 6 weeks.

Transformation: Frozen cells were thawed on ice and a 40 µl aliquot was transferred to a pre-cooled 0.2 cm electroporation cuvette (Bio-Rad Laboratories Ltd.) One µl of plasmid DNA (2-10 ng) was mixed with the cell suspension on ice and an electric pulse applied immediately using a Gene Pulser™ with Pulse Controller unit (Bio-Rad). Highest transformation efficiencies (up to 8 X 10^5) were obtained at a field strength of 12.5 kV/cm, a capacitance of 25 µF and resistors of 400 or 600 ohms in parallel with the sample, giving time constants of 8-12 msec (Fig. 1). The cells were immediately transferred to 1 ml YMB or TY and shaken at 29° for 3 h. Aliquots of 10 µl or 100 µl were plated on YMB agar containing appropriate antibiotics and incubated for 3 d at 29°.

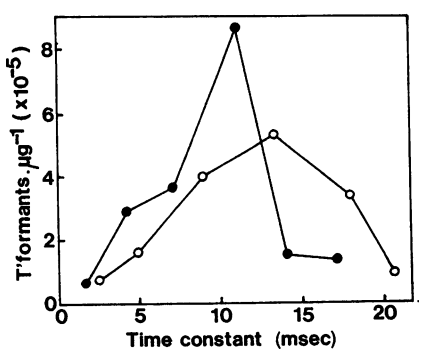

Figure 1. Effect of field strength and pulse length on efficiency of transformation of *A. tumefaciens*. 7.5 ng of pBI121 DNA (3) was used for each electroporation and the pulse lengths were varied by selecting resistors of 100 to 1000 ohms at field strengths of 10 kV/cm (O) and 12.5 kV/cm (●). Similar results were obtained with *A. rhizogenes*.

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