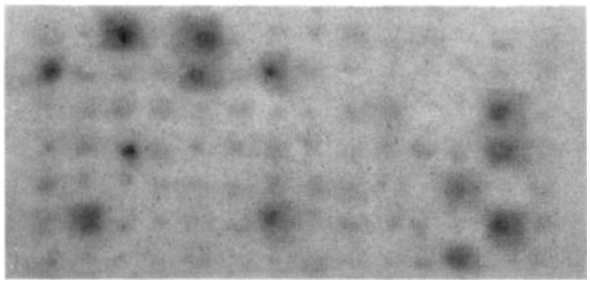

FIGURE 3 Colony-hybridization analysis using ^{32}P-labeled oligonucleotide as the probe. After transformation as shown in Figure 1, 98 transformants were respotted on a square Whatman 3MM filter paper and examined by colony-hybridization analysis as previously described[4].

plasmid as well as the wild-type plasmid, it is important to isolate the mutant plasmid by retransformation. From one of the retransformed cells, plasmid DNA was purified and sequenced, which confirmed the mutation caused by the synthetic oligonucleotide (data not shown).

The present method for oligonucleotide-directed site-specific mutagenesis is very simple and efficient, and can be applied to any gene cloned in a plasmid vector. However, the use of the versatile high expression pIN-II vectors is particularly convenient because the expression of the cloned gene can be regulated by a *lac* inducer, and the unique XbaI, EcoRI, HindIII, BamHI, KpnI, and SalI sites[8] can be used to remove the portion of the cloned gene to be mutagenized.

The yield of the mutant in the present study (13%) is comparable to that reported with the use of an M13 vector (6 to 45%[5]). A major factor in obtaining a better yield is the efficiency of construction of circular DNA structures (DNA-a and DNA-b in Fig. 1). We have found that heat denaturation followed by gradual cooling was much better than alkaline denaturation followed by neutralization as employed by Oostra et al.[9]. Another important factor is the elimination of ampicillin-resistant background transformants caused by DNA-c and DNA-d (Fig. 1). Although the number of ampicillin-resistant transformants due to DNA-d was negligible, those due to DNA-c may be substantial. This could be eliminated by the addition of a SmaI linker during the ligation step followed by SmaI digestion before transformation.

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ERRATUM

Due to an editorial error the wrong photograph appeared above the legend for Figure 5 in the paper titled "A New Chimeric Gene as a Marker for Plant Transformation: The Expression of *Escherichia coli* β-Galactosidase in Sunflower and Tobacco Cells" by Georgia Helmer, Malcolm Casadaban, Michael Bevan, Lucy Kayes, and Mary-Dell Chilton in the June issue (**2:**520–527). The correct figure and legend are printed below.

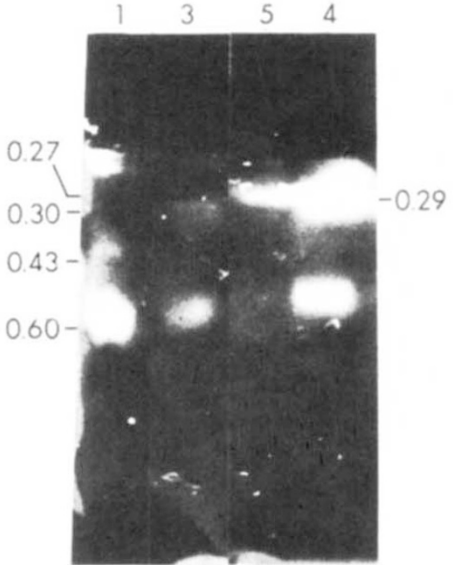

FIGURE 5 Native polyacrylamide gel electrophoresis of extracts of engineered plant *Nicotiana tabacum SR-1* tissues photographed after staining with fluorogenic β-galactosidase substrate 4-MU-galactosidase in pH 7 buffer. Migration is from the top to the bottom as photographed. The gel is 7.5% acrylamide in phosphate-citrate buffer. Lane 5 contains a standard of *E. coli* β-galactosidase. Lanes 1, 3 and 4 were loaded with 58 micrograms of protein from extracts of transformed cloned tobacco tissue. Lane 4 is a control of pGHlac$^-$ extract to which was added 100 units of *E. coli* β-galactosidase just subsequent to centrifugation. Lane 3 contains an extract of cloned pGHlac$^+$ tissue. Lane 1 contains extract of pGHlac$^-$ tissue. Ferritin, a colored protein of native molecular weight ca. 450,000, served as a visible marker. Relative mobilities, calculated by dividing the migration distance of the unknown by the migration distance of the bromphenol blue tracking dye, are given in the figure as R_f values. Ferritin has an R_f of 0.27. *E. coli* β-galactosidase monomer has an R_f of 0.29; the pGHlac$^+$ fusion protein has an R_f of 0.3. Two bands of endogenous β-galactosidase activity are seen, one major at R_f 0.60, and one minor at R_f 0.43.