



Fig. 1. (C) PCR amplification products obtained using  $\beta$ -glucuronidase gene-specific sense (GUSS400: 5'-GGTGGGAAAGCGCGTTACAAG-3') and antisense (GUSA1599: 5'-GTTTACGCGTTGCTTCCGCCA-3') primers. The PCR program used was the same as for A. DNAs used as substrate in each lane were: -, reaction mix without added DNA; +, 10 pg  $\beta$ -glucuronidase-containing plasmid DNA in 100 ng wild-type T309 DNA; 1-4, bialaphos-resistant transgenic rice cotransformed with *bar* and  $\beta$ -glucuronidase chimeric genes; 5 and 6, wild-type T309 plants. Lane M contains BRL 1 kb ladder-size standards.

been cut to completion by running a small aliquot on a gel before running the gel to be blotted (*see Note 7*).

2. Cast a 0.7% agarose gel in 1X Tris-acetate buffer. A higher concentration gel can be used if resolution of smaller fragments is desired.
3. Add 5-6  $\mu$ L loading buffer to each DNA sample, load the gel and electrophorese. Recirculate the buffer, since 1X TAE has little buffering capacity. (In 1X TAE buffer, the dye front will migrate about 10 cm if run at 0.8 V/cm for ~16 h, i.e., 23 V in a BRL H5 gel box [for running 11  $\times$  14 cm gels].)
4. Carefully transfer gel to a glass baking dish. Cut ~2-3 mm off each side and the bottom to remove the menisci. Remove the upper portion of the gel by cutting through the wells. Horizontally shave the meniscus off the upper surface of the gel at each well. Cut the top right corner off the gel to make orientation easy.
5. Add ethidium bromide solution (200-300 mL) and stain DNA by gentle agitation on an orbital shaker for 20-30 min.
6. Place the stained gel on a UV light box (302 nm), align a ruler with the wells beside a lane with size standards and photograph.
7. Introduce single-strand breaks into the high-mol-wt DNA with UV light (254 nm) (*see Note 8*).