Luciferase reporter gene cassettes for plant gene expression studies

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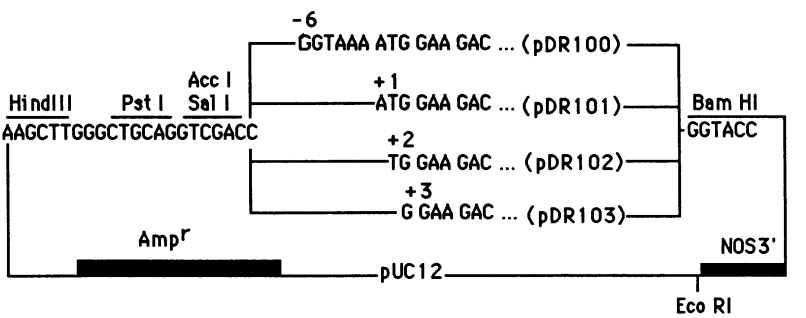
To facilitate our analyses of plant gene expression (1,2), we have constructed a set of vectors containing the polyadenylation site of the nopaline synthase gene linked to deletion mutants of the reporter gene luciferase (3). The luciferase clone pDO472 was resected from its unique Sal I site 90bp upstream of the ATG codon by exonuclease III/mung bean nuclease treatment, and synthetic Sal I linkers were attached. The inserts of clones ending in the A, T, and G of the initiation codon were removed by digestion with Sal I and Bam HI, and inserted into the plasmid pNOS3' (a 250bp Mbo I fragment engineered into the Sma I site of pUC12). The resulting vectors (pDR101, 102, 103) can be used for constructing translational in frame fusions with promoters having Hind III, Pst I, Sal I, or Acc I ends. Fusion proteins have been produced which retain luciferase activity, indicating that the amino terminus of luciferase can be altered without deleterious effects (Baughman and Howell, personal communication). Additionally, the plasmid pDR100 has been constructed for transcriptional fusions to luciferase, and this clone ends at postion -6 relative to the ATG codon. Chimeric genes constructed with these cassettes can be used to transform plants (either stably or transiently, refs 4,5), and gene expression can be evaluated rapidly and quantitatively by employing the sensitive luciferase assay (6).



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