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pPAV, a promoter analysis vector for *Dictyostelium discoideum*

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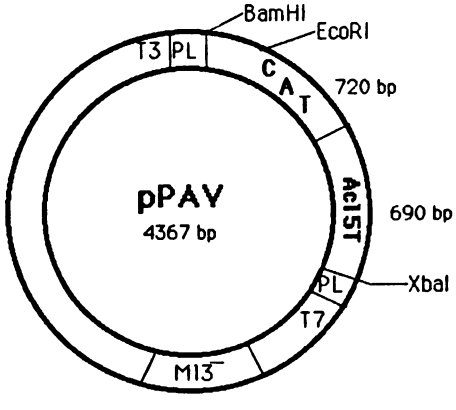
Over the past several years, reliable methods for DNA-mediated transformation of Dictyostelium discoideum have been developed (1,2). The vectors which have been developed carry the gene conferring neomycin resistance, and this drug is used to select for transformants. In order to facilitate the analysis of promoter regions and to define cis acting elements involved in gene expression in this organism, a vector termed pPAV (promoter analysis vector) has been constructed. This vector, after insertion of suspected promoter regions, can be introduced into Dictyostelium by cotransformation(3) with one of the existing neoR containing vectors (1,2). As shown below, the chloramphenicol acetyl transferase (CAT) gene serves in a reporter capacity for evaluation of promoter efficiencies. Dictyostelium possesses no detectable CAT activity during vegetative growth or during development. A termination site for Dictyostelium RNA polymerase II derived from the actin 15 gene (4) is positioned downstream of the CAT gene. The features of the parental plasmid, Bluescript KS- of Stratagene, allow for the facile manipulation of promoter sequences. These include the T7 and T3 promoters for strand specific probe generation, appropriate polylinker regions (PL) for the generation of exonuclease III deletion series to facilitate sequence analysis as well as defining cis elements, ability to readily generate linker-scanner type mutations (3) with 7 basepair replacements, and the presence of the M13 origin allows isolation of single stranded material for site directed mutagenesis. Finally, CAT specific oligonucleotides can be utilized to determine mRNA levels and can be used in primer extension experiments in order to map transcription start sites.



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