A vector for expressing GAL4(1−147) fusions in mammalian cells

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Transcriptional activator proteins bind to specific enhancer elements in eukaryotic promoters, and stimulate transcription of nearby genes (1). Separable functional elements of activator proteins confer specific DNA binding, transcriptional activation, and regulation (1). These functional domains are remarkably flexible, and readily tolerate fusion to heterologous proteins (2,3). Thus, a currently popular technique for studying activating and regulatory elements of transcriptional activators involves fusion to the DNA binding domain of the well characterized yeast GAL4 protein. These fusion proteins can be assayed for activity on promoters bearing GAL4 binding sites (2-4).

To accommodate such experiments, we have developed a useful vector for the construction and expression of GAL4(1-147) fusions in mammalian cells. GAL4(1-147) binds specifically to DNA, but does not activate transcription (5). The vector pSG424 (Figure 1A) contains the coding sequence of GAL4(1-147) immediately followed by a multiple cloning site. Following the polylinker sequence are translational termination codons in three reading frames. Transcripts encoding the fusion proteins are expressed from the SV40 early promoter and terminated within a fragment containing the SV40 polyadenylation signal. The polylinker encodes a 16 amino acid peptide with a net charge of 0. We cannot detect transcriptional activation by the GAL4(1-147)+16 protein produced by pSG424, although the protein is easily detected in immunoprecipitates from COS-1 cells. We have fused several activating domains to GAL4(1-147) using this vector; each was expressed at similar levels, and we found no evidence of instability of the fusion proteins.

This vector and the complete nucleotide sequence are available upon request.

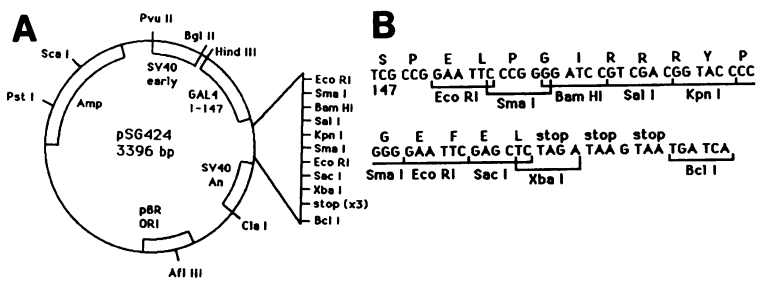

Figure 1. A) pSG424 vector for the construction and expression of GAL4(1-147) fusions in mammalian cells. The plasmid contains the SV40 ori/early promoter region fused to the coding sequence for GAL4(1-147), followed immediately by a polylinker and translational stop codons. Transcripts are terminated within an SV40 DNA segment containing a polyA signal. B) Sequence of the polylinker region from amino acid codon 147 of GAL4, and the peptide encoded by the polylinker.

REFERENCES

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