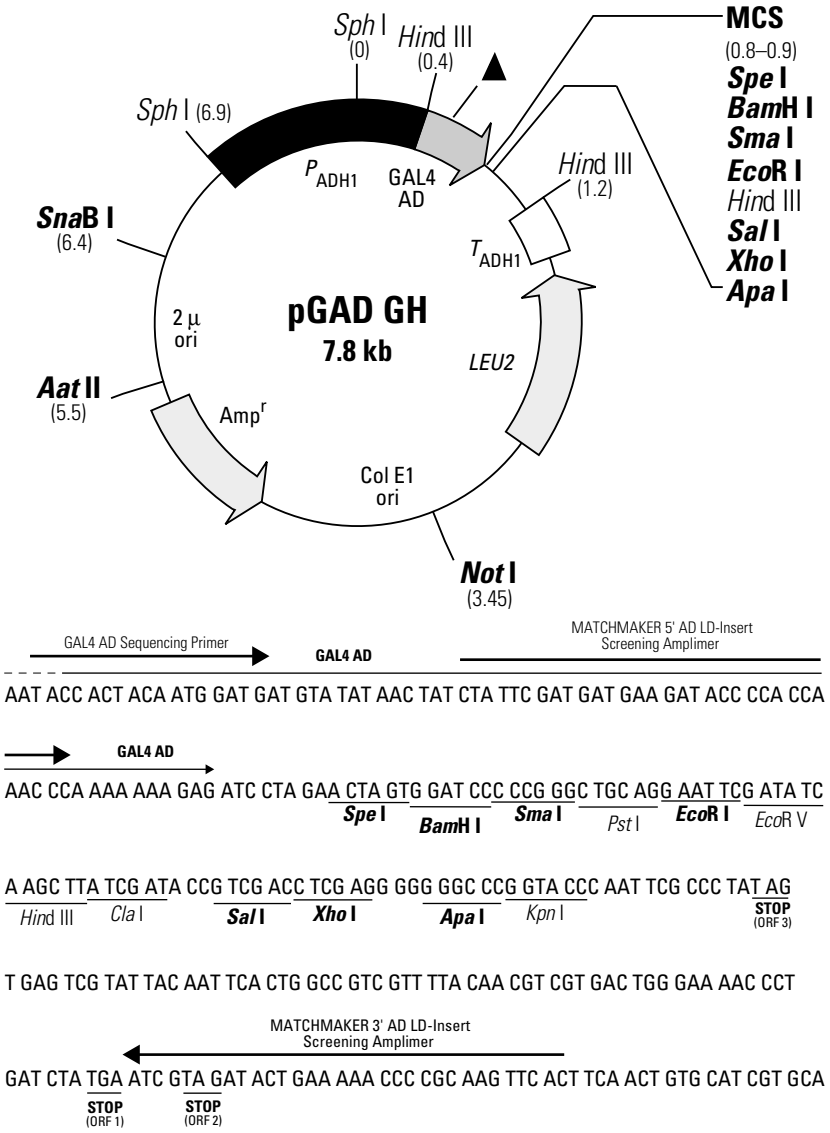

Restriction Map and Multiple Cloning Site (MCS) of pGAD GH. Unique restriction sites are in bold.

Description:

pGAD GH generates a hybrid protein that contains the sequences for the GAL4 activation domain (amino acids 768–881). pGAD GH has unique restriction sites located in the MCS region at the 3'-end of the open reading frame for the activation domain sequence. For the construction of a hybrid protein, the gene encoding the protein of interest (or a collection of cDNAs) is ligated into the MCS in the correct orientation and with the correct reading frame such that a fusion protein is generated. The fusion protein is expressed at high levels in yeast host cells from the constitutive ADH1 promoter; transcription is terminated at the ADH1 transcription termination signal. The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences that have been added to the activation domain sequence from a heterologous source (Chien *et al.*, 1991). pGAD GH is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae*. It carries the *bla* gene (for ampicillin resistance in *E. coli*) and the *LEU2* nutritional marker that allow yeast auxotrophs carrying pGAD GH to grow on limiting synthetic medium lacking Leu.

REFERENCES

1. Bartel, P. L.,*et.al.* (1993) Using the two-hybrid system to detect protein-protein interactions. In *Cellular Interactions in Development: A Practical Approach*, D. A. Hartley, Ed., Oxford University Press, Oxford; pp 153–179.
2. Chien, C. T., *et.al.* (1991) The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Natl. Acad. Sci. USA* **88**:9578–9582.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.