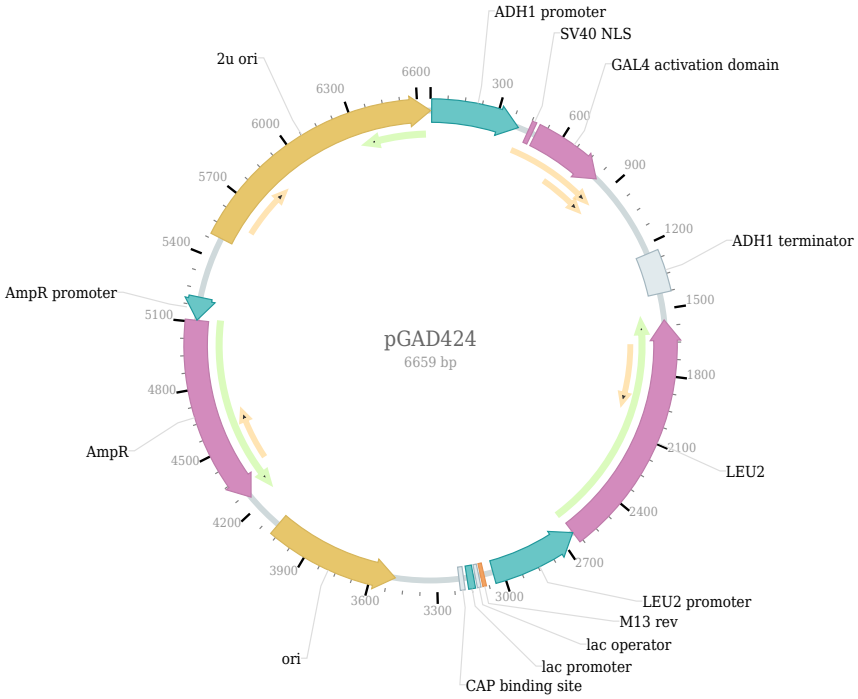
pGAD424

Vector ID	V001273
Antibiotic Resistance	Ampicillin
Length	6659 bp
Vector Type	Yeast one hybrid systems
Host	Yeast

Product Information

Replication Origin	ori
Promoter	LEU2
Comment	pGAD424 generates a hybrid protein that contains the sequences for the GAL4 activation domain (AD; a.a. 768–881) (1). pGAD424 has unique restriction sites located in the MCS region at the 3'- end of the open reading frame for the GAL4 AD 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 in 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 AD sequence from a heterologous source (2). pGAD424 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 pGAD424 to grow on limiting synthetic medium lacking Leu.

Vector Map



Plasmid Resuspension Protocol

1. Centrifuge at 5,000×g for 5 min.
2. Carefully open the tube and add sterile water to dissolve the DNA: add 20 µl for 5 µg plasmid, and 100 µl for 100 µg plasmid.
3. Close the tube and incubate for 10 minutes at room temperature.
4. Briefly vortex the tube and then do a quick spin to concentrate the liquid at the bottom. Speed is less than 5000×g.
5. Store the plasmid at -20 ℃.