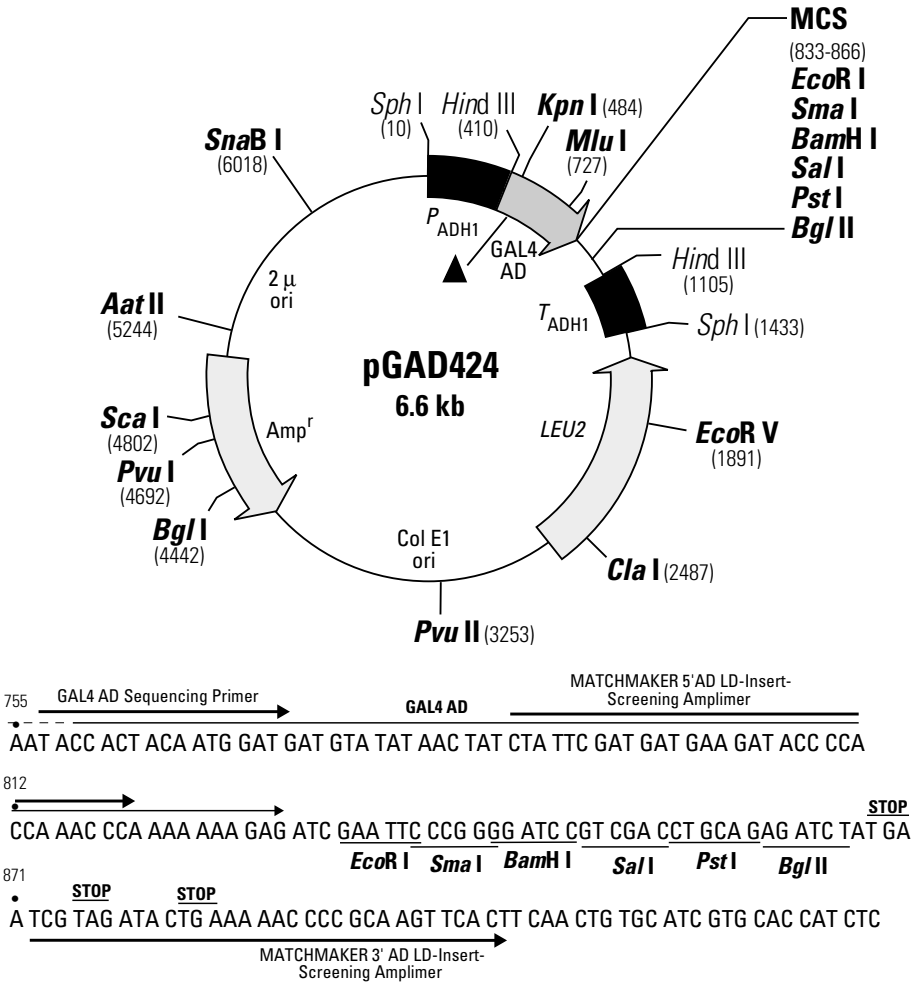
Vector Information

pGAD424 AD Vector Information PT1022-5

GenBank Accession #: U07647 Catalog #K1605-1



Restriction map and multiple cloning site (MCS) of pGAD424. Unique restriction sites are in bold.

Description:

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.

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(PR81584)

Location of features

- Promoter fragment carrying the truncated *S. cerevisiae ADH1* promoter: 10–406
- GAL4 activation domain (AD) polypeptide
  start codon (ATG): 422–424; stop codon: 875–877; GAL4 codons 768–881: 491–829
  SV40 T-antigen nuclear localization signal: 452–472
- Multiple cloning site: 834–866
- Translation stop codons: 868–870, 875–877 & 882–884
- Transcription termination signal
  Fragment carrying the *S. cerevisiae ADH1* terminator: 1105–1432
- *LEU2* coding sequences: start codon (ATG): 2640–2638; stop codon: 1548–1546
- Col E1 plasmid replication origin: 3457–4100
- Ampicillin resistance gene
  Promoter: –35 region: 5178–5173; –10 region: 5155–5150
  Transcription start point: 5143
  Ribosome binding site: 5120–5116
  β-lactamase coding sequences:
  Start codon (ATG): 5108–5106; stop codon: 4250–4248
  β-lactamase signal peptide: 5108–5040
  β-lactamase mature protein: 5039–4251

Primer locations

- MATCHMAKER 5' AD LD-Insert Screening Amplimer (#9103-1): 757–773
- MATCHMAKER 3' AD LD-Insert Screening Amplimer (#9103-1): 908–889

Propagation in *E. coli*

- Suitable host strains: DH5a, DH10 & other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: 15–20

Propagation in *S. cerevisiae*

- Suitable host strains: Y187(α), Y190(a), SFY526(a), CG1945(a), or HF7c(a)
- Selectable marker: *LEU2*
- *S. cerevisiae* origin: 2 μ

References

1. Bartel, P. L., *et al.* (1993) In *Cellular Interactions in Development: A Practical Approach* (Oxford University Press, Oxford) pp. 135–179.
2. Chien, C. T., *et al.* (1991) *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.