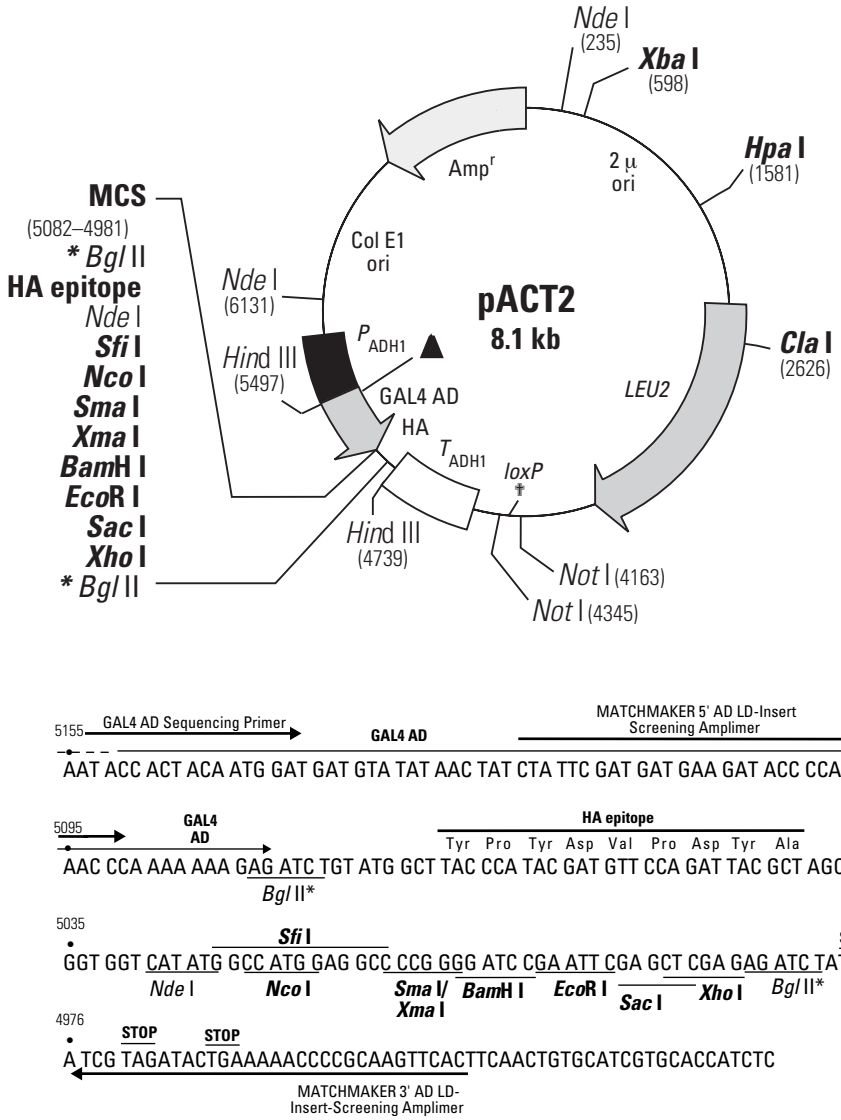
Vector Information

pACT2 AD Vector Information PT3022-5

GenBank Accession No.: U29899. Catalog 638822



Restriction Map and Multiple Cloning Site (MCS) of pACT2 AD. Unique restriction sites are in bold.

Description:

pACT2 generates a fusion of the GAL4 AD (amino acids 768–881), an HA epitope tag, and a protein of interest (or protein encoded by a cDNA in a fusion library) cloned into the MCS in the correct orientation and reading frame. pACT2, which is derived from pACT (1), contains a unique *Eco*R I site in the MCS. The hybrid protein is expressed at high levels in yeast host cells from the constitutive ADH1 promoter (*P*); transcription is terminated at the ADH1 transcription termination signal (*T*). The protein is targeted to the yeast nucleus by the nuclear localization sequence from SV40 T-antigen which has been cloned into the 5' end of the GAL4 AD sequence (2). pACT2 is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae* and carries the *bla* gene, which confers ampicillin resistance in *E. coli*. pACT2 also contains the *LEU2* nutritional gene that allows yeast auxotrophs to grow on limiting synthetic media. Transformants with AD/library plasmids can be selected by complementation by the *LEU2* gene by using an *E. coli* strain that carries a *leuB* mutation (e.g., HB101).

Note: The *Sfi* I and *Sma* I sites in the MCS tend to compress during sequencing.

(PR8X956)

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Location of features:

- 2µ origin of replication: 1–2055
- LEU2 coding sequences
  - Start codon (ATG): 2474–2476
  - Stop codon: 3566–3568
- Lox sites: Lox 1: 4268–4327; Lox 2: 4367–4412
- Transcription termination signal
  - Fragment carrying the *S. cerevisiae* ADH1 terminator: 4415–4742
  - Translation stop codon: 4970–4972
- Multiple cloning site: 4927–5079
- Hemagglutinin (HA) epitope: 5042–5068
- GAL4 activation domain polypeptide
  - Start codon (ATG): 5486–5488
  - GAL4 codons 768–881: 5081–5419
  - SV40 T-antigen nuclear localization signal: 5424–5478
- Promoter fragment carrying the *S. cerevisiae* ADH1 promoter: 5504–5901
- pBR322 plasmid replication origin: 6336–6979
- Ampicillin resistance gene
  - Promoter: –35 region: 8052–8057; –10 region: 8029–8034
  - Transcription start point: 8022
  - Ribosome binding site: 7995–7999
  - β-lactamase coding sequences:
  - Start codon (ATG): 7985–7987
  - Stop codon: 7127–7129
  - β-lactamase signal peptide: 7919–7987
  - β-lactamase mature protein: 7130–7918

Primer locations:

- MATCHMAKER 5' LD-Insert Screening Amplimer: 5122–5091
- MATCHMAKER 3' LD-Insert Screening Amplimer: 4943–4974
- GAL4 AD Sequencing Primer: 5153–5137

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pBR322
- 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* replication origin: 2µ
- Copy number: multiple copy

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.