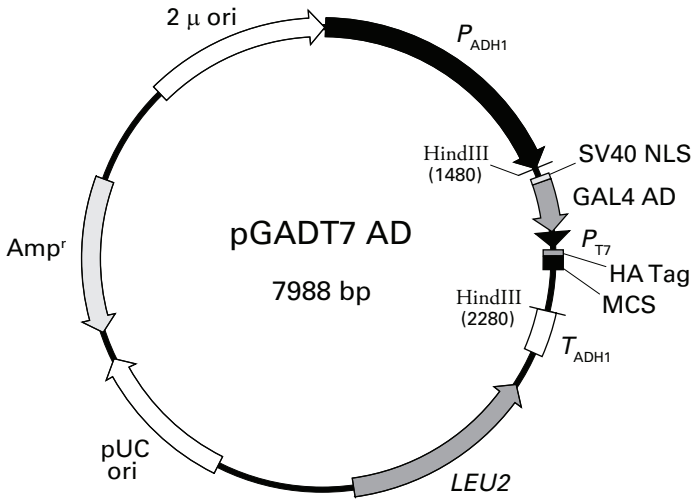
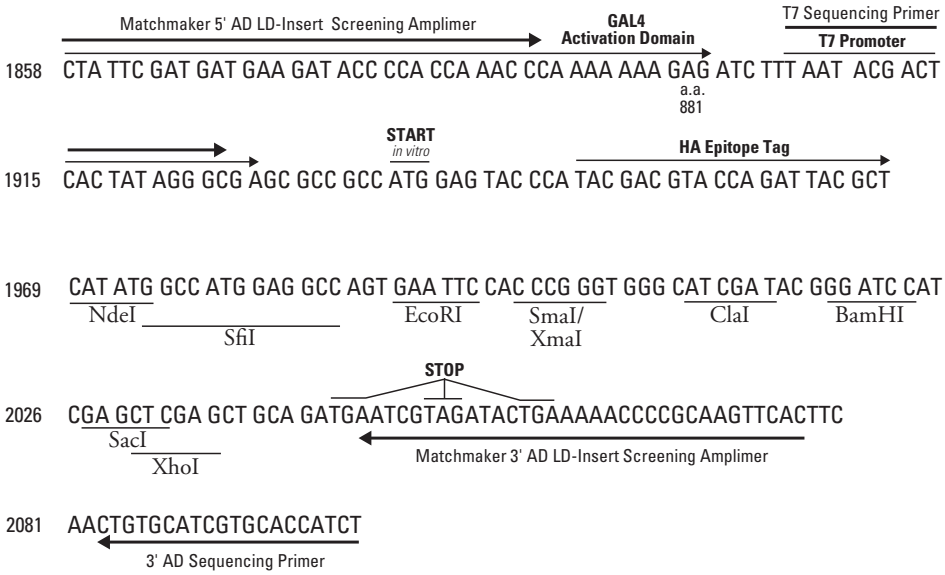
Vector Information

pGADT7 AD Vector Information

PT3249-5

Cat. Nos. 630442
630489
630491





pGADT7 AD Vector Map and Multiple Cloning Site (MCS).

Description

pGADT7 AD is a yeast expression vector that is designed to express a protein of interest fused to a GAL4 activation domain (AD; amino acids 768–881). Transcription of the GAL4 AD fusion is driven by the constitutively active *ADH1* promoter (P_{ADH1}), and is terminated at the *ADH1* transcription termination signal (T_{ADH1}). The GAL4 AD fusion contains an N-terminal SV40 nuclear localization signal (SV40 NLS; 1) that targets the protein to the yeast nucleus, and a hemagglutinin epitope tag (HA Tag), located between the GAL4 AD and the protein of interest, that allows the protein to be easily detected with HA-tag antibodies.

The T7 promoter (P_{T7}), located just upstream of the HA tag sequence, allows *in vitro* transcription and translation of the HA-tagged protein of interest (without the GAL4 AD and the SV40 NLS). pGADT7 AD replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 □ ori, respectively. The vector also contains an ampicillin resistance gene (Amp^r) for selection in *E. coli* and a *LEU2* nutritional marker for selection in yeast.

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Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Use

pGADT7 AD is the AD Cloning Vector provided in Clontech's Matchmaker™ Gold Yeast One- and Two-Hybrid Screening Systems (Cat. Nos. 630491 and 630489, respectively). The vector allows the generation of GAL4 AD fusion proteins from either a gene of interest or a cDNA library. **Important:** Genes must be cloned into the MCS so that they are in-frame with the GAL4 AD and HA tag coding sequences. The vector can also be used as a negative "prey" control for the One-Hybrid System.

GAL4 AD/HA-tagged fusion proteins expressed by the vector can be detected with either our GAL4 AD Monoclonal Antibody (Cat. No. 630402) or our HA-Tag Polyclonal Antibody (Cat. No. 631207). Note: *In vitro* transcription/translation from the T7 promoter, located between the GAL4 AD and HA tag sequences, produces an HA-tagged protein that lacks the GAL4 AD. Such proteins can be detected by the HA-Tag Polyclonal Antibody, but not the GAL4 AD Monoclonal Antibody.

Location of features

- P_{ADH1} (full-length *S. cerevisiae ADH1* promoter): 7–1479
- GAL4 AD (GAL4 activation domain with SV40 Nuclear Localization Signal [NLS])
  SV40 NLS: 1501–1557
  GAL4 (amino acids 768–881): 1561–1899
- P_{T7} (T7 RNA polymerase promoter): 1905–1927
- HA Tag (hemagglutinin epitope tag): 1942–1968
- MCS (multiple cloning site): 1969–2041
- T_{ADH1} (*S. cerevisiae ADH1* Terminator): 2280–2605
- *LEU2* coding sequences: 2723–3814 (complementary)
- pUC ori (pUC replication origin): 4581–5418
- Amp^r (ampicillin resistance gene): 5574–6434 (complementary)
- 2 μ ori (Yeast 2 μ replication origin): 6998–7988

Location of primers

- T7 Sequencing Primer: 1905–1925
- 3' AD Sequencing Primer: 2102–2083
- Matchmaker 5' AD LD-Insert Screening Amplimer (Cat. No. 630433): 1858–1889
- Matchmaker 3' AD LD-Insert Screening Amplimer (Cat. No. 630433): 2078–2046

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Propagation in *S. cerevisiae*

- Suitable host strains: Y1HGold, Y2HGold, AH109(*MAT***a**), Y187(*MAT*α), Y190(*MAT***a**), SFY526(*MAT***a**), CG1945(*MAT***a**), HF7c(*MAT***a**)
- Selectable marker: *LEU2*
- *S. cerevisiae* origin: 2 μ

Reference

1. Chien, C.T., Bartel, P. L., Sternglanz, R. & Fields, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**:9578–9582.

Note: The vector sequence was 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.