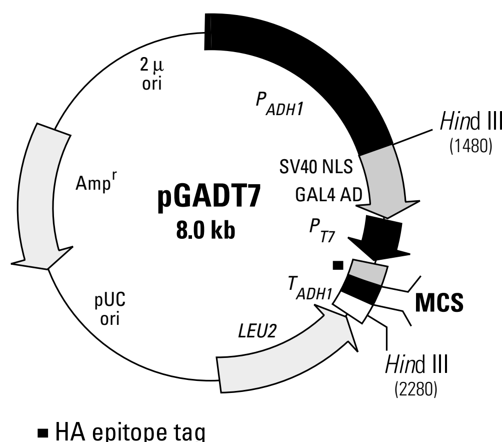
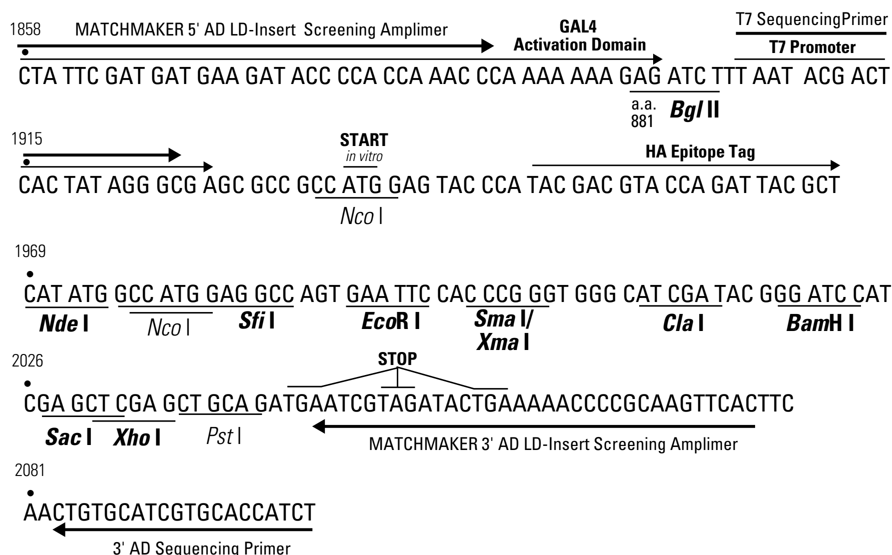
GenBank Accession #: Submission in progress. Catalog # K1612-1





Restriction Map and Multiple Cloning Site (MCS) of pGADT7. Unique restriction sites are in bold.

Description:

The pGADT7 vector expresses proteins fused to amino acids 768–881 of the GAL4 activation domain (AD). In yeast, fusion proteins are expressed at high levels from the constitutive *ADH1* promoter (P_{ADH1}); transcription is terminated at the *ADH1* transcription termination signal (T_{ADH1}). The fusion protein is targeted to the yeast nucleus by the SV40 nuclear localization sequences that have been added to the activation domain sequence (1). pGADT7 also contains the T7 promoter, an HA epitope tag, and a MCS. pGADT7 replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 μ ori, respectively. The vector carries Amp^r for selection in *E. coli* and the *LEU2* nutritional marker for selection in yeast.

Use:

pGADT7 is the AD Vector included with MATCHMAKER Two-Hybrid System 3. The MCS of pGADT7 has unique restriction sites in frame with the 3'-end of the GAL4 AD for constructing a fusion protein with either a protein of interest or a fusion protein library. The bait protein is also expressed

as a fusion to a hemagglutinin (HA) epitope tag. HA-tagged proteins can be identified with antibodies raised to this common epitope, eliminating the need to generate specific antibodies to new proteins. The T7 promoter is used for *in vitro* transcription and translation of the epitope tagged fusion protein and also provides a binding site for sequencing using the T7 Sequencing Primer. Note that the AD is not expressed during the *in vitro* transcription and translation reactions.

The *Nco* I and *Pst* I sites may be used to shuttle inserts from pGADT7 into pGBKT7, the MATCHMAKER Two-Hybrid System 3 DNA-BD Vector. The MCS in pGADT7 is compatible with those in pMyc-CMV and pHA-CMV, CLONTECH's epitope tagged mammalian expression vector set (#K6003-1). As a result, the target gene can be shuttled into these vectors in order to confirm protein interactions *in vivo*.

Location of features:

- Full-length *S. cerevisiae ADH1* promoter (P_{ADH1}): 7–1479
- GAL4 AD polypeptide with SV40 Nuclear Localization Signal (NLS)
  - NLS: 1501–1557
  - GAL4 amino acids 768–881: 1561–1899
- T7 RNA polymerase promoter: 1905–1927
- HA epitope tag: 1942–1968
- Multiple Cloning Sites: 1969–2041
- Transcription termination signal
  - Fragment carrying the *S. cerevisiae ADH1* terminator (T_{ADH1}): 2280–2605
- *LEU2* coding sequences: 3814–2723
- pUC plasmid replication origin: 4581–5418
- Ampicillin resistance gene: 6432–5575
- 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 (#9103-1): 1858–1889
- MATCHMAKER 3' AD LD-Insert Screening Amplimer (#9103-1): 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: Y187(α), Y190(a), SFY526(a), CG1945(a), HF7c(a), or AH109(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.

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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.

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