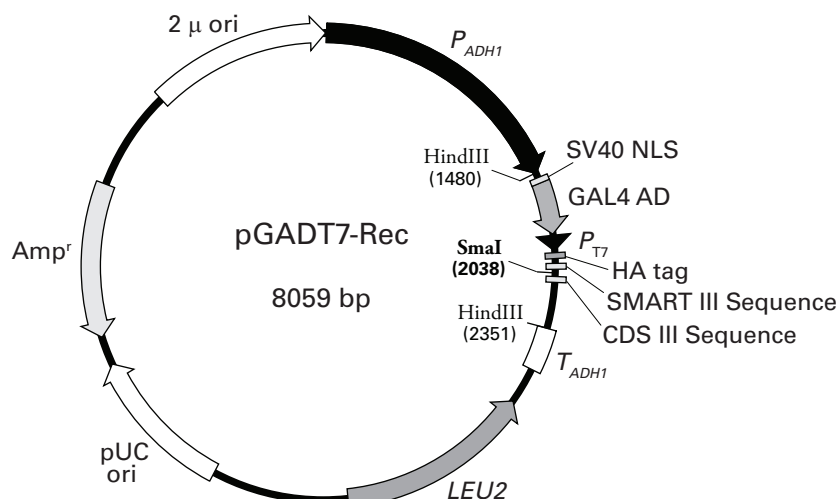


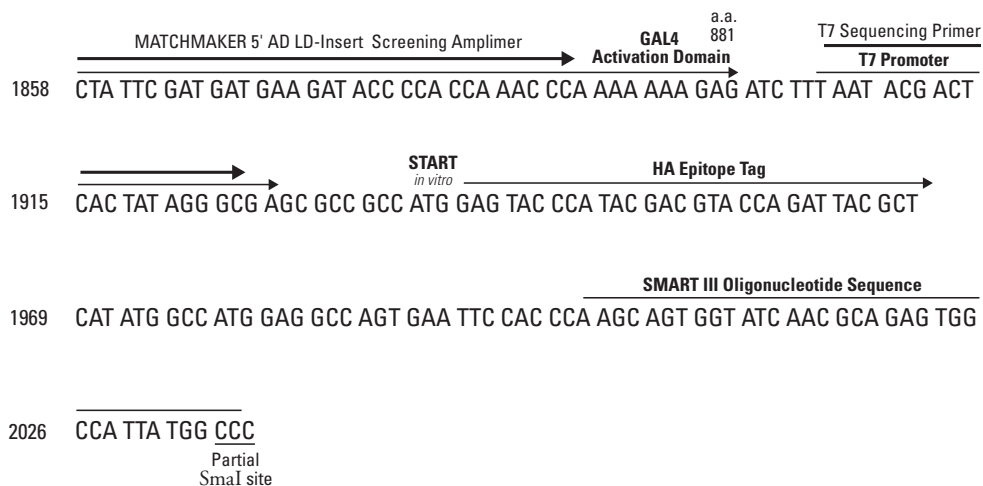
pGADT7-Rec Vector Information

PT3530-5

Sold as part of Cat. Nos. 630490 & 630491



SMART™ III terminus



CDS III terminus

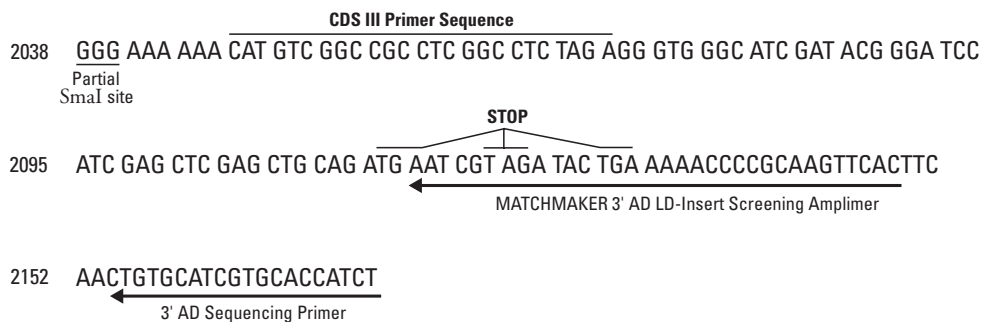


Figure 1. pGADT7-Rec Vector Map and Cloning Site. A unique restriction site (SmaI) is shown in bold. Both the Make Your Own "Mate & Plate" Library System and the Matchmaker™ Gold Yeast One-Hybrid System (Cat. Nos. 630490 and 630491, respectively) contain the SmaI-linearized form of this vector, the form used for recombination-mediated cloning in yeast.

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Description

In yeast, pGADT7-Rec expresses a protein of interest as a GAL4 activation domain (GAL4 AD) fusion. Transcription starts with the constitutive *ADH1* promoter (P_{ADH1}) and ends with the *ADH1* termination signal (T_{ADH1}). The GAL4 AD sequence includes the SV40 nuclear localization signal (SV40 NLS; 1) so that fusions translocate to the yeast nucleus. GAL4 AD fusions also contain a hemagglutinin epitope tag (HA tag) for easy identification with Clontech's HA-Tag Polyclonal Antibody (Cat. No. 631207).

The T7 promoter in pGADT7-Rec allows *in vitro* transcription and translation of the hemagglutinin (HA)-tagged fusion protein. It also provides a binding site for the T7 Sequencing Primer. In its circular form, pGADT7-Rec 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-Rec is engineered for the construction of GAL4 AD/cDNA libraries by homologous recombination in yeast (Figure 2). Libraries made with this vector can be used for Matchmaker™ Gold One- and Two-Hybrid Screening.

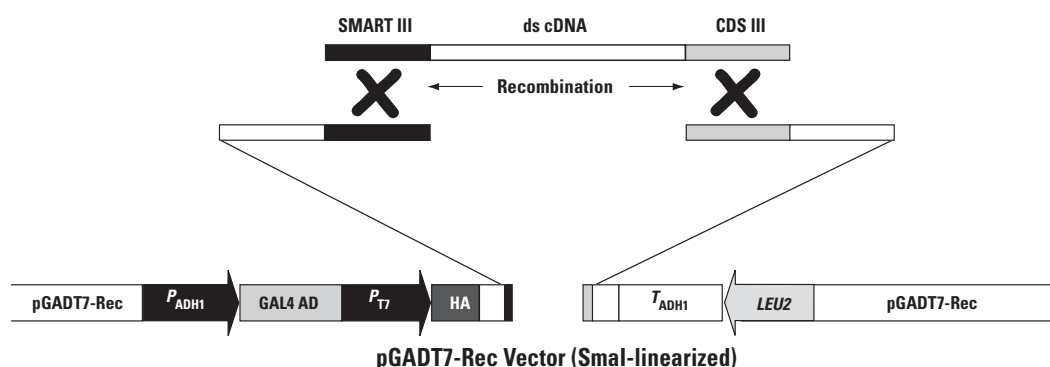


Figure 2. Cloning cDNA into pGADT7-Rec by homologous recombination *in vivo*. The ends of the SmaI-linearized vector are homologous to Clontech's SMART™ III Oligonucleotide and CDS III Primer, used in the Matchmaker cDNA synthesis protocol (Figure 1).

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 AD (amino acids 768–881): 1561–1899
- P_{T7} (T7 RNA polymerase promoter): 1905–1927
- HA tag (hemagglutinin epitope tag): 1942–1968
- SMART III Oligonucleotide sequence: 2001–2036
- CDS III Primer sequence: 2047–2071
- T_{ADH1} (*S. cerevisiae ADH1* Terminator): 2351–2676
- *LEU2* coding sequences: 2794–3885 (complementary)
- pUC ori (pUC replication origin): 4652–5489
- Amp^r (ampicillin resistance gene): 5646–6503 (complementary)
- 2 μ ori (yeast 2 μ replication origin): 7069–8059

Location of primers

- T7 Sequencing Primer: 1905–1927
- 3' AD Sequencing Primer: 2173–2154
- Matchmaker 5' AD LD-Insert Screening Amplimer (Cat. No. 630433): 1858–1889
- Matchmaker 3' AD LD-Insert Screening Amplimer (Cat. No. 630433): 2149–2117

Propagation in *E. coli*

- Suitable host strains: DH5 α , DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100 μ g/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(MATa), Y187(MAT α), Y190(MATa), SFY526(MATa), CG1945(MATa), HF7c(MATa)
- 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.

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