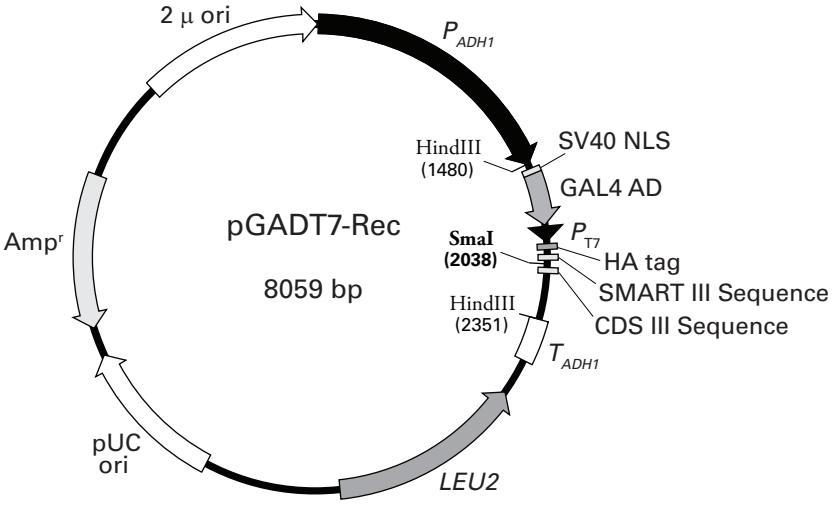
Vector Information

pGADT7-Rec Vector Information PT3530-5

Sold as part of Cat. Nos. 630490 & 630491



SMART™ III terminus

MATCHMAKER 5' AD LD-Insert Screening Amplimer — GAL4 Activation Domain — a.a. 881 — T7 Sequencing Primer — T7 Promoter

1858 CTA TTC GAT GAT GAA GAT ACC CCA CCA AAC CCA AAA AAA GAG ATC TTT AAT ACG ACT

START *in vitro* — HA Epitope Tag

1915 CAC TAT AGG GCG AGC GCC GCC ATG GAG TAC CCA TAC GAC GTA CCA GAT TAC GCT

SMART III Oligonucleotide Sequence

1969 CAT ATG GCC ATG GAG GCC AGT GAA TTC CAC CCA AGC AGT GGT ATC AAC GCA GAG TGG

2026 CCA TTA TGG CCC

Partial SmaI site

CDS III terminus

CDS III Primer Sequence

2038 GGG AAA AAA CAT GTC GGC CGC CTC GGC CTC TAG AGG GTG GGC ATC GAT ACG GGA TCC

Partial SmaI site

STOP

2095 ATC GAG CTC GAG CTG CAG ATG AAT CGT AGA TAC TGA AAAACCCCGCAAGTTCACTTC

MATCHMAKER 3' AD LD-Insert Screening Amplimer

2152 AACTGTGCATCGTGCACCATCT

3' AD Sequencing Primer

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.

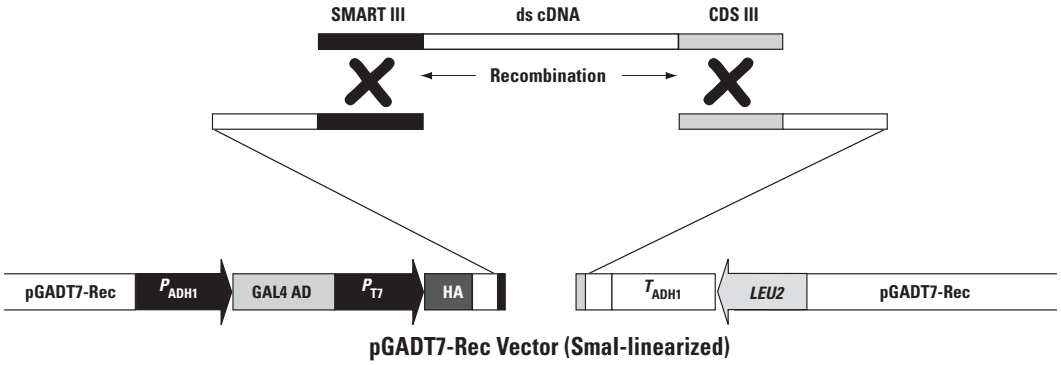

Figure 2. Cloning cDNA into pGADT7-Rec by homologous recombination *in vivo*. The ends of the SmaI-linearized vector are homologous to Clontech's SMART™ III Oligonucleotide and CDS III Primer, used in the Matchmaker cDNA synthesis protocol (Figure 1).

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: 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.