pGADT7-Rec Vector

Catalog No.

(Not sold separately)

Sold as a part of 630490 & 630491

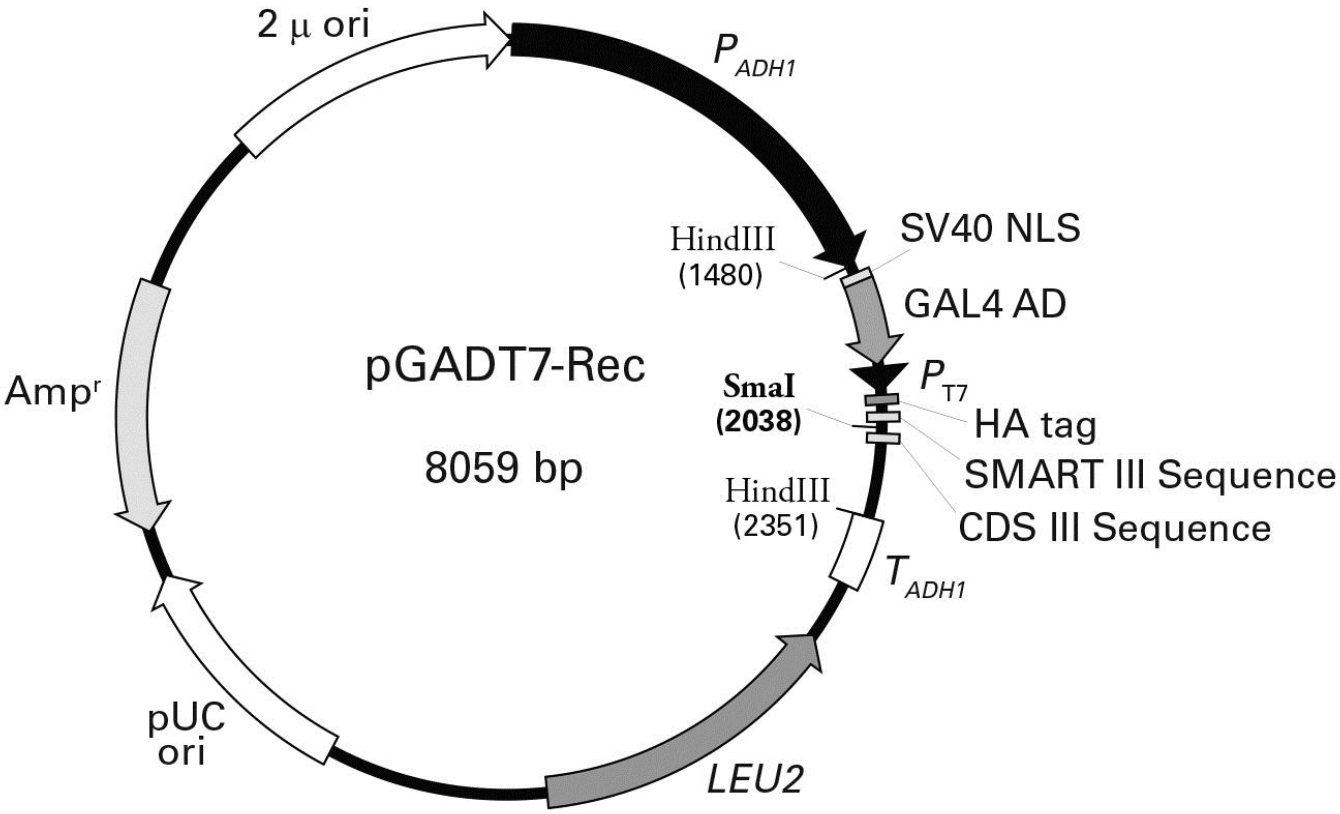

Figure 1. pGADT7-Rec Vector map. A unique restriction site (SmaI) is shown in bold. Both the Make Your Own "Mate & Plate" Library System and the Matchmaker® Gold Yeast One-Hybrid System (Cat. Nos. 630490 and 630491, respectively) contain the SmaI-linearized form of this vector, the form used for recombination-mediated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

Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada 800.662.2566 | Asia Pacific +1.650.919.7300 | Europe +33.(0)1.3904.6880 | Japan +81.(0)77.565.6999

(102016)

Vector Map

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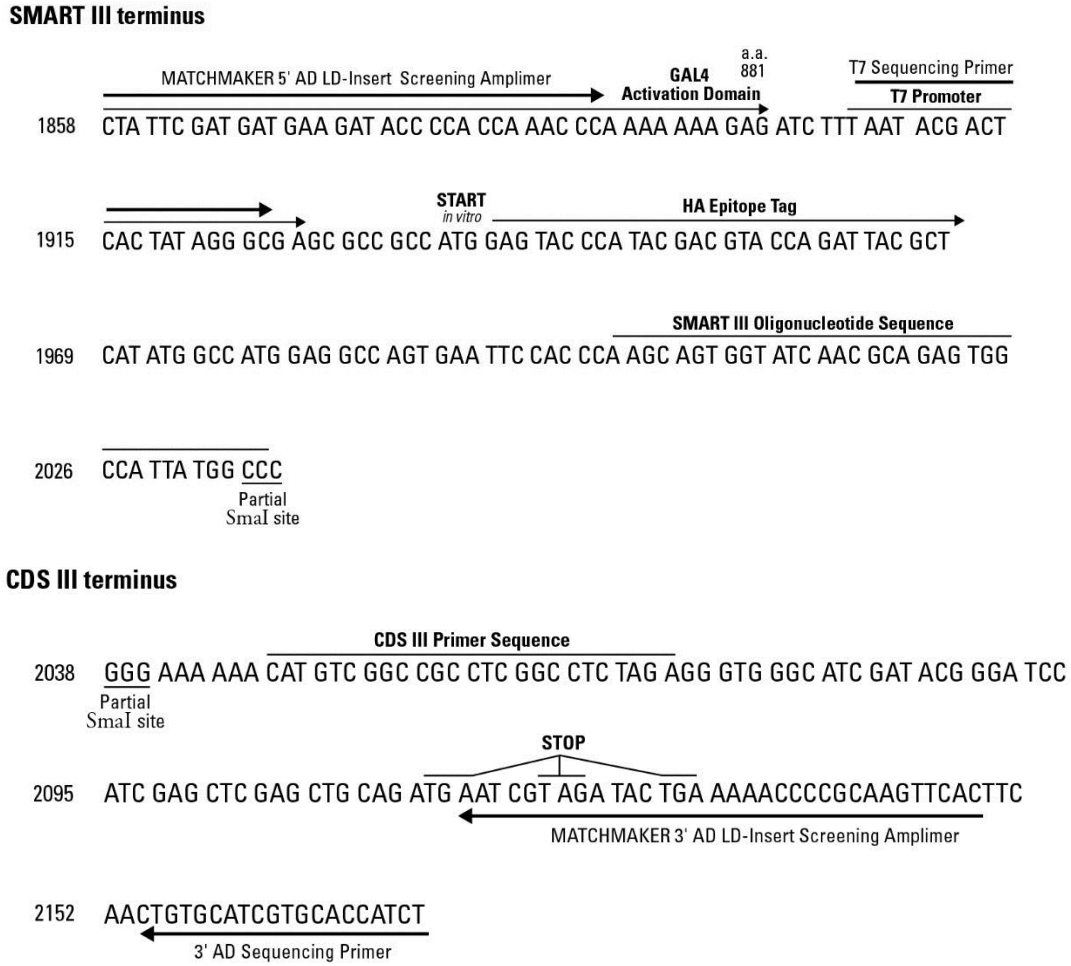

Figure 2. pGADT7-Rec Vector cloning site.

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.

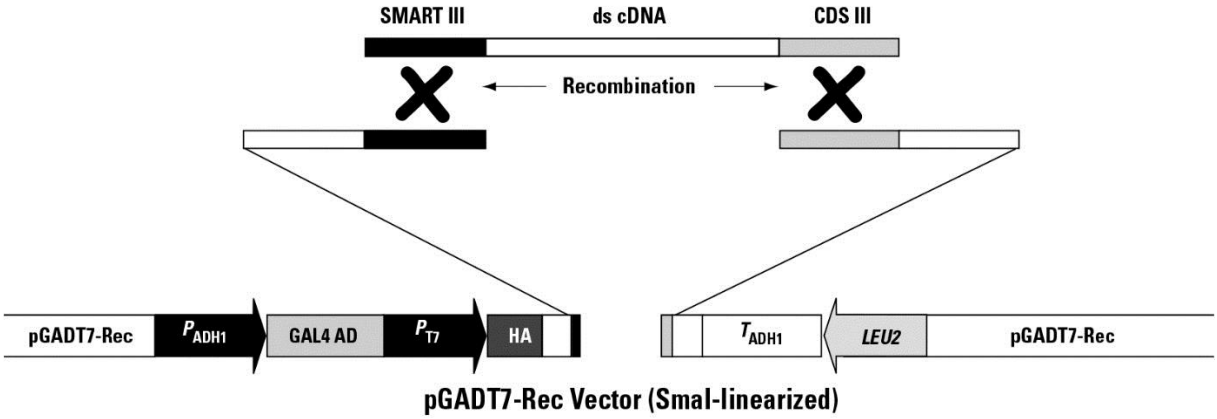

Figure 3. Cloning cDNA into pGADT7-Rec by homologous recombination *in vivo*. The ends of the SmaI-linearized vector are homologous to our SMART® III Oligonucleotide and CDS III Primer, used in the Matchmaker cDNA synthesis protocol (Figure 2).

Vector Map

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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)
- 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 strain: 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

NOTE: The vector sequence was compiled from information in the sequence databases, published literature, and other sources, together with partial sequences we obtained. This vector has not been completely sequenced.

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This document has been reviewed and approved by the Quality Department.