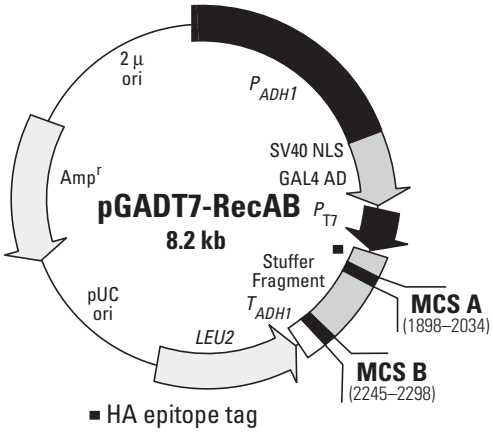
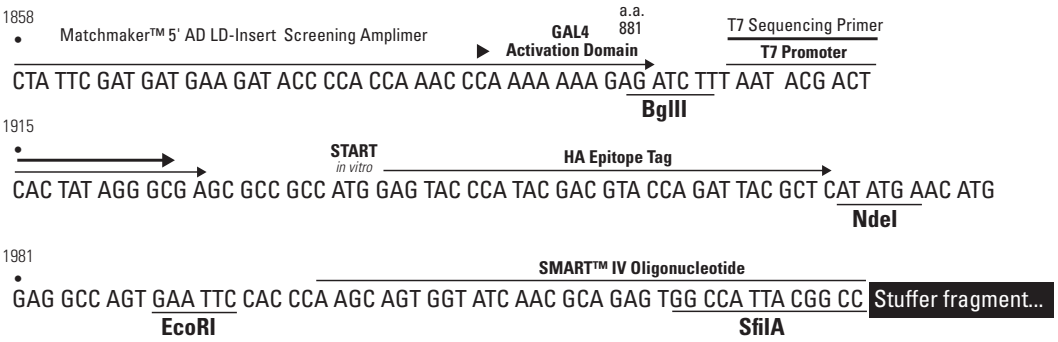


MCS A



MCS B

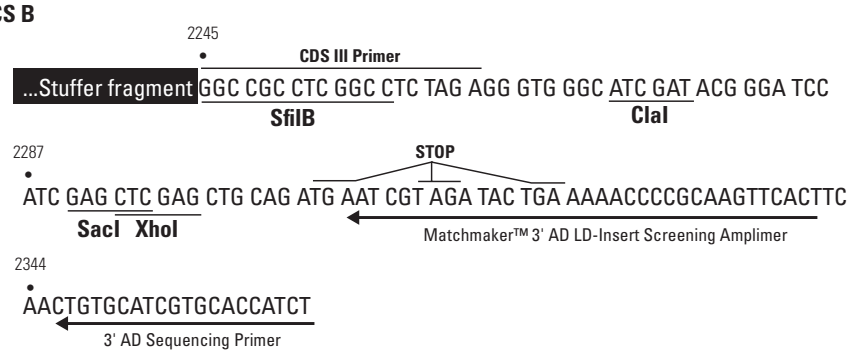


Figure 1. Restriction Map and Cloning Sites of pGADT7-RecAB Vector. Unique restriction sites are in bold. The stuffer fragment is replaced by a cDNA insert when the library is constructed.

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Description

In yeast, pGADT7-RecAB 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 (HA) epitope tag for easy identification with our HA-Tag Polyclonal Antibody (Cat. No.631207).

pGADT7-RecAB contains a T7 promoter for *in vitro* transcription and translation of the hemagglutinin (HA)-tagged fusion protein. (Note that the GAL4 AD is not transcribed and translated from the T7 promoter.) The T7 promoter can also be used as a binding site for sequencing with a T7 Sequencing Primer. In its circular form, pGADT7-RecAB replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 μ ori, respectively. The vector carries an ampicillin resistance gene (Amp^r) for selection in *E. coli*, and the *LEU2* nutritional marker for selection in yeast.

The vector contains two, non-identical *Sfi* I sites (denoted *Sfi* IA and *Sfi* IB in Figure 1) so that GAL4 AD/cDNA fusion libraries can be constructed *in vitro* using the SMART™ method shown in Figure 2. The SMART IV and CDS III oligos used in this reaction incorporate the corresponding *Sfi* I sites into the ends of the cDNAs. *Sfi* I sites are extremely rare in mammalian DNA, so nearly all SMART cDNAs remain intact during the subsequent *Sfi* I digestion. Only the ends of the cDNA are cut. Following the *Sfi* I digestion, the cDNA is ligated to the *Sfi* I-digested pGADT7-RecAB Vector. Note that the *Sfi* I cloning method, as shown in Figure 2, maintains the directionality of the cDNA.

Use

pGADT7-RecAB is a high-copy, high-level expression vector that can be used in yeast to identify and characterize novel protein-protein (two-hybrid) interactions. Libraries constructed in pGADT7-RecAB can be screened using any GAL4-based two-hybrid system, but we strongly recommend using Clontech Matchmaker™ Two-Hybrid Systems, which supplies the protocols and many of the essential reagents needed for two-hybrid screening.

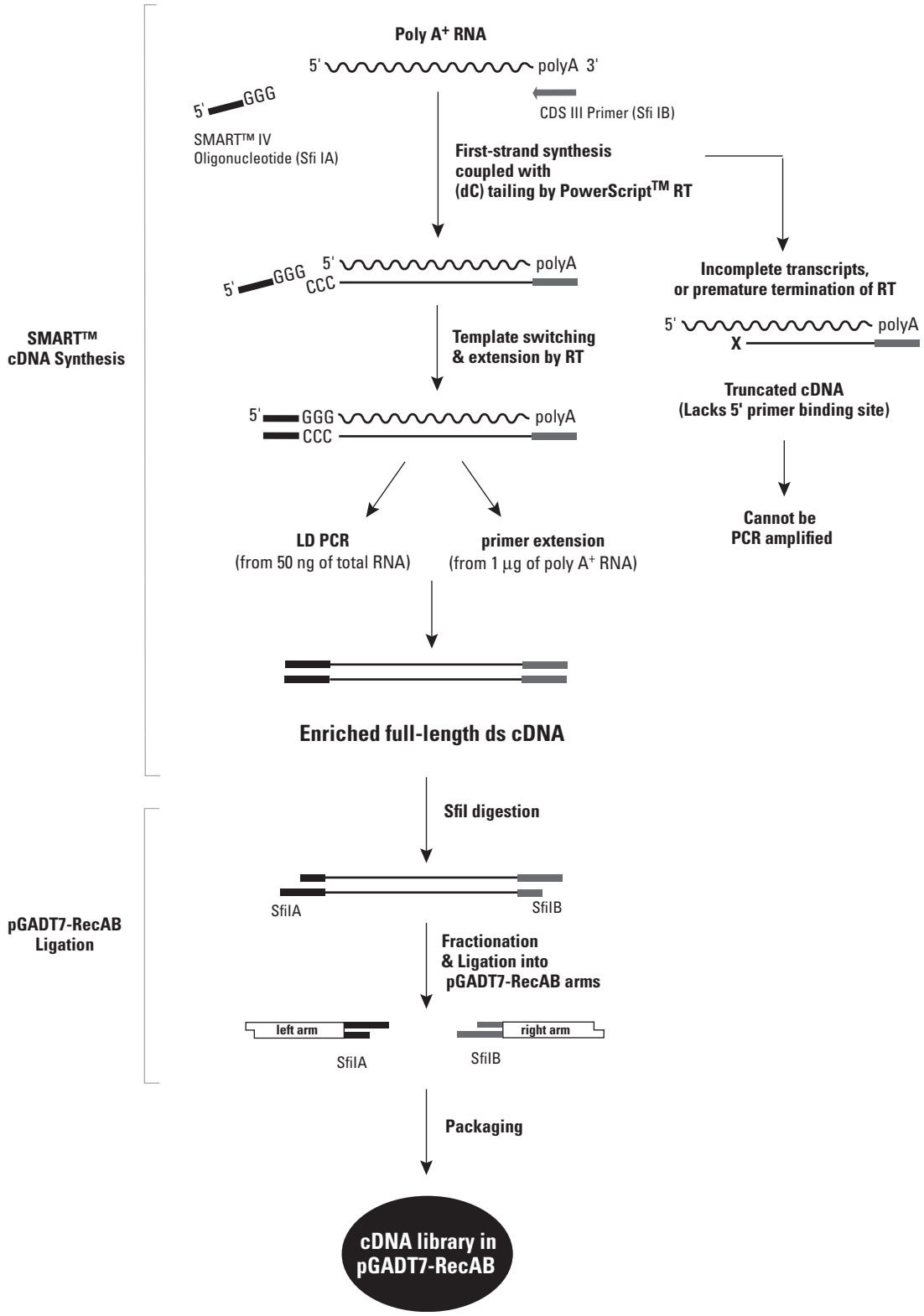

Figure 2. Constructing GAL4 AD/cDNA libraries in pGADT7-RecAB Vector. To learn more about the SMART cDNA Library Construction method, please see our User Manual (PT3000-1), available at www.clontech.com/clontech.

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
- MCS A: 1898–2036
- T7 RNA polymerase promoter: 1905–1927
- HA epitope tag: 1939–1968
- SMART IV Oligonucleotide sequence: 2001–2036
- Stuffer fragment: 2037–2244
- CDS III Primer sequence: 2245–2263
- MCS B: 2245–2298
- Transcription termination signal
  Fragment carrying the *S. cerevisiae ADH1* terminator (T_{ADH1}): 2543–2868
- *LEU2* coding sequences: 4077–2986
- pUC plasmid replication origin: 4844–5681
- Ampicillin resistance gene: 6695–5838
- Yeast 2 μ replication origin: 7261–8251

Location of primers

- T7 Sequencing Primer: 1905–1925
- 3' AD Sequencing Primer: 2365–2346
- Matchmaker 5' AD LD-Insert Screening Amplimer (Cat. No. 630433): 1858–1889
- Matchmaker 3' AD LD-Insert Screening Amplimer (Cat. No. 630433): 2340–2308

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: 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 attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.

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