Vector pBDC (Fig.1) was constructed from the *TRP1* vector by changing the sequence at –9 to –4 with respect to the Gal4-DBD ATG initiator codon from TCCTGA to TCGCGA. This introduces a unique NruI restriction endonuclease site 6bp upstream of the DBD. The resulting pBDC plasmid is slightly more versatile than the parent vector pOBD2 in that it can be used to position the Gal4p DBD at the either the N-terminus or the C-terminus of any open reading frame (ORF). Generation of N-terminal DBD fusions is through *in vivo* recombination yeast between Nco1+PvuII-cut vector pBDC (or pOBD2) and the ORF as a linear fragment (the latter generated by two sequential PCR amplifications). Generation of C-terminal DBD fusions also involves recombination in yeast between linearised pBDC and the ORF of interest as a PCR product. However in this case pBDC is linearised with Nru1 (Fig. 1) and the ORF-encoding fragment that is generated by the two sequential PCR amplifications has different sequence extensions. To generate C-terminal DBD fusions the initial PCR reaction uses primers that have 3' sequence homology to the ORF, but 5' sequence homology to the vector (forward primer gcttgaagcaagcctcg ATG *** *** *** *** *** ***; reverse primer: cagtagcttcatctttcg*** *** *** *** *** *** *** (ATG *** *** etc. corresponding to the codon positions of the ORF of interest and the lower case letters the sequences of the pBDC vector)). The product of this first PCR reaction is then used as the template in a second round of PCR that uses primers completely homologous to regions flanking the Nru1 site of the vector (forward primer: caactccaagcttgaagcaagcctcgatg; reverse primer: cgatagaagacagtagcttcatctttcg).

Vector pADC (Fig. 1) was created from the *LEU2* vector pOAD by substituting TCGCGA for the AAGCTT sequence of the latter plasmid at –13 to –8 relative to the Gal4p AD. This introduces a Nru1 restriction site. pADC can be used to position the Gal4 AD at the either the N-terminus or the C-terminus of an ORF. N-terminal AD fusions are generated by *in vivo* recombination in yeast between this vector cut with Nco1+PvuII and the ORF as a PCR product (generated by two sequential PCR amplifications using primers with the sequence extensions described by). Generation of fusions where the AD is positioned at the C-terminus of an ORF involves *in vivo* recombination between Nru1-linearised pADC and the ORF-encoding fragment that is again generated by two sequential PCR amplifications. The first of these PCR reactions uses primers that have 3' sequence homology to the ORF, but 5' sequence homology to the vector (forward primer: agcatacaatccaag ATG *** *** *** *** *** *** *** *; reverse primer: cgctttatccatctttgcaaaggc *** *** *** *** *** *** *** *). The product of this first PCR reaction is then used as template in a second round of PCR, using primers homologous to the regions adjacent to the pADC Nru1 site (forward primer: caagctataccaagcatacaatccaagatg, reverse primer: ggaattaattccgctttatccatctttgcaaaggc).

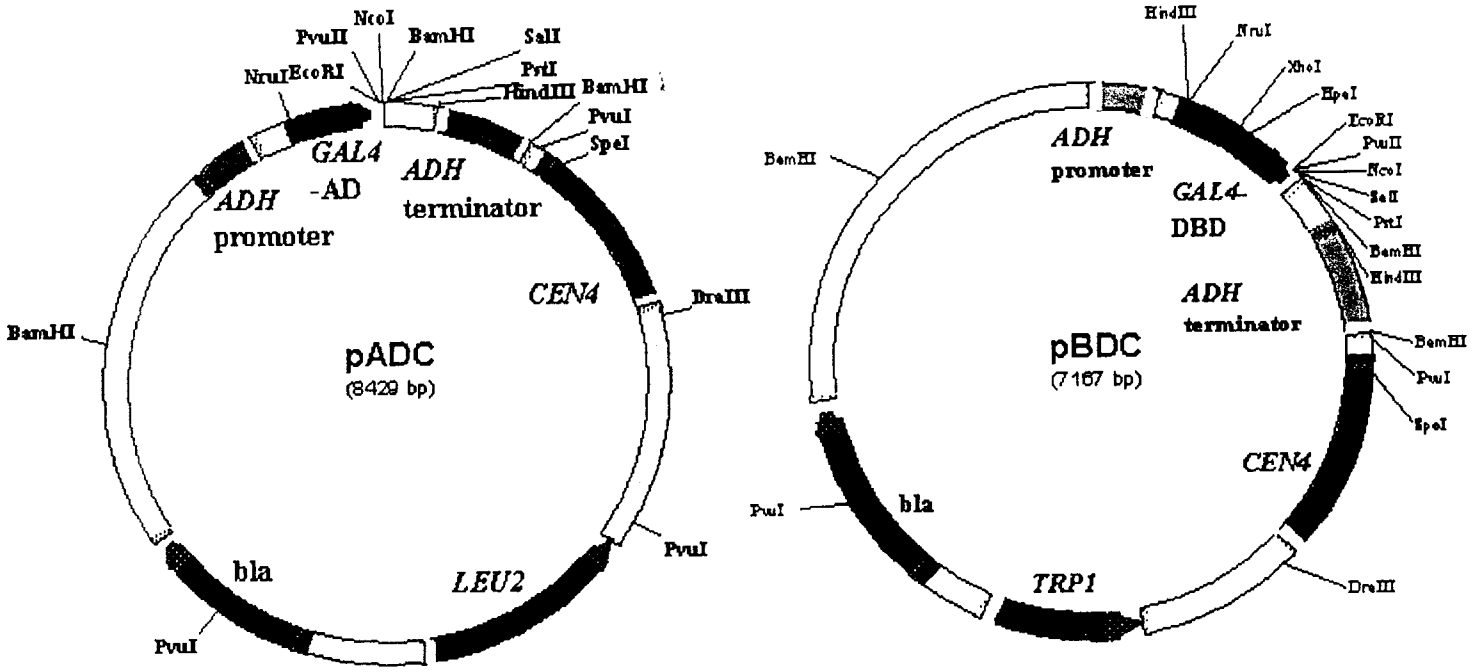
NcoI
PvuII
BamHI
SalI
NruI
EcoRI
PstI
HindIII
BamHI
PvuI
SpeI
GAL4
-AD
ADH
promoter
ADH
terminator
CEN4
DraIII
BamHI
pADC
(8429 bp)
PvuI
bla
LEU2
PvuI
HindIII
NruI
XhoI
EpeI
EcoRI
PstI
NcoI
SalI
PstI
BamHI
HindIII
BamHI
ADH
promoter
GAL4-
DBD
ADH
terminator
pBDC
(7167 bp)
BamHI
PvuI
SpeI
CEN4
PvuI
bla
TRP1
DraIII

Figure 1