

Figure 1— Schematic illustration of bipartite geminivirus genome organization. The locations of coding and non-coding DNA sequences in the A and B genome components are shown. Open reading frames (ORFs) and their direction of transcription are depicted by arrows. Major non-coding regions on each DNA component contain a sequence of near-identity, termed the common region (CR) depicted by thick lines. In addition, there are unique non-coding intergenic sequences between the *CR* and the *ARI*, *BLI*, and *BRI* ORFs, which are designated *ARi*, *BLi*, and *BRi*, respectively, and are depicted by thin lines.

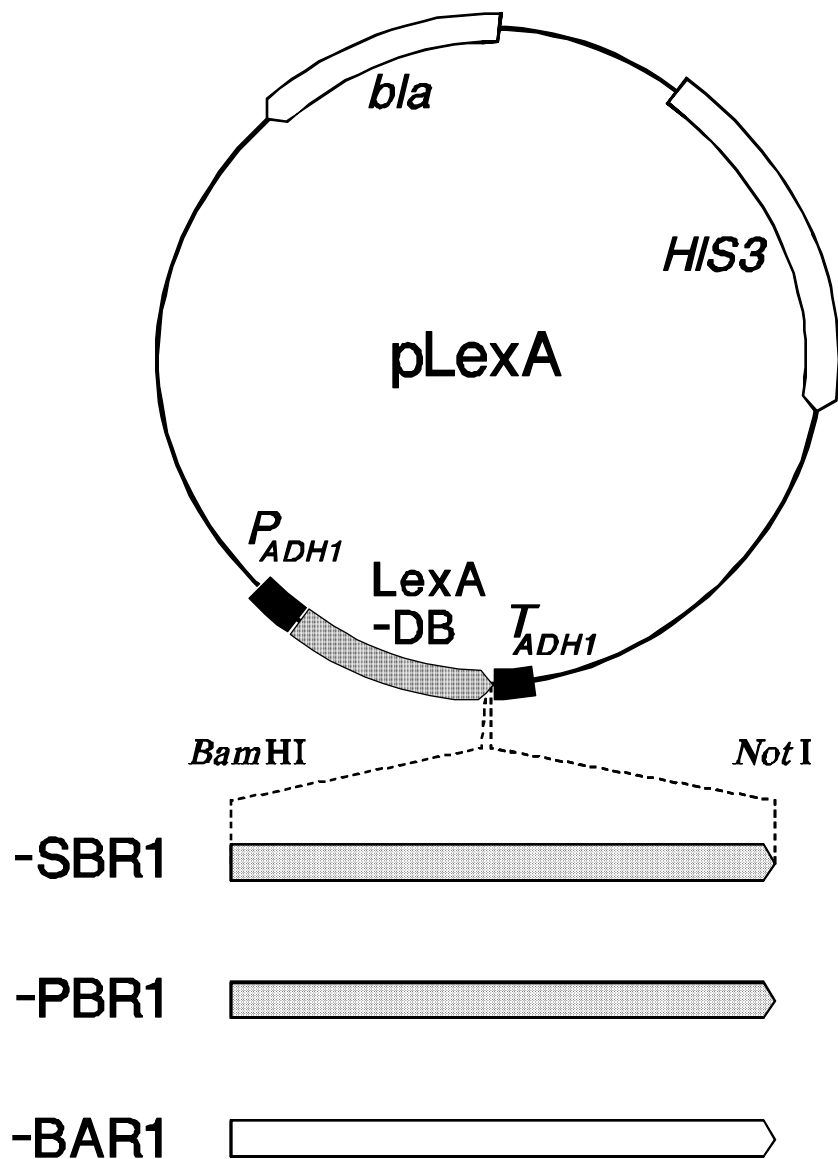


Figure 2 — Schematic of yeast two-hybrid ‘bait’ vector pLexA and its derivatives. The plasmid contains replication origins (not shown) that function in *E. coli* and yeast, as well as selectable markers *bla* (ampicillin resistance in *E. coli*) and *HIS3* (histidine prototrophy in yeast). The DNA-binding domain of the *E. coli* LexA protein (LexA-DB) is expressed constitutively in yeast under control of the *ADH1* promoter (P_{ADH1}) and transcription terminator (T_{ADH1}). pLexA derivatives contained geminivirus ORFs inserted in-frame with LexA-DB by ligation between the polylinker *Bam*HI and *Not*I sites. Bait plasmids were designated according to the viral ORF expressed: pLexA-SBR1 (SLCV *BR1*), pLexA-PBR1 (TPV *BR1*), pLexA-BAR1 (BGMV *AR1*).

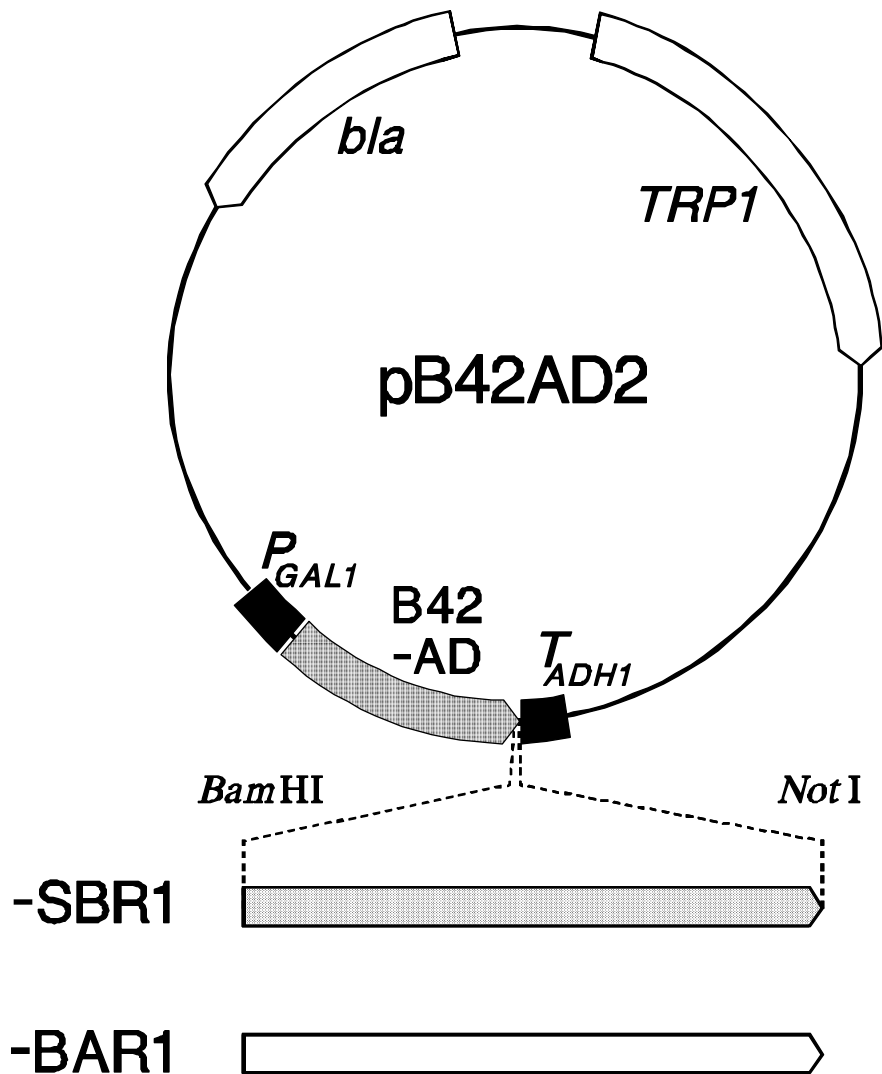
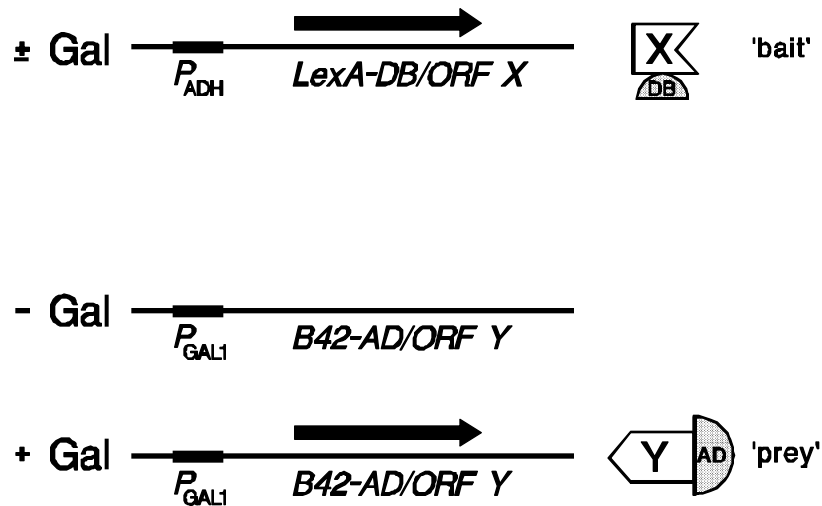


Figure 3 — Schematic of yeast two-hybrid ‘prey’ vector pB42AD2 and its derivatives. The plasmid contains replication origins (not shown) that function in *E. coli* and yeast, as well as selectable markers *bla* (ampicillin resistance in *E. coli*) and *TRP1* (tryptophan prototrophy in yeast). The B42 activation domain (B42-AD), plus a nuclear localization signal and hemagglutinin epitope tag (not shown), is expressed in yeast under control of the galactose-inducible *GAL1* promoter (P_{GAL1}) and the *ADH1* transcription terminator (T_{ADH1}). pB42AD2 derivatives contained geminivirus ORFs inserted in-frame with B42-AD by ligation between the polylinker *Bam*HI and *Not*I sites. Prey plasmids were designated according to the viral ORF expressed: pB42AD-SBR1 (SLCV *BR1*), pB42AD-BAR1 (BGMV *AR1*).

(A) Expression of 'bait' and 'prey' fusion proteins



(B) Detection of two-hybrid fusion protein interactions

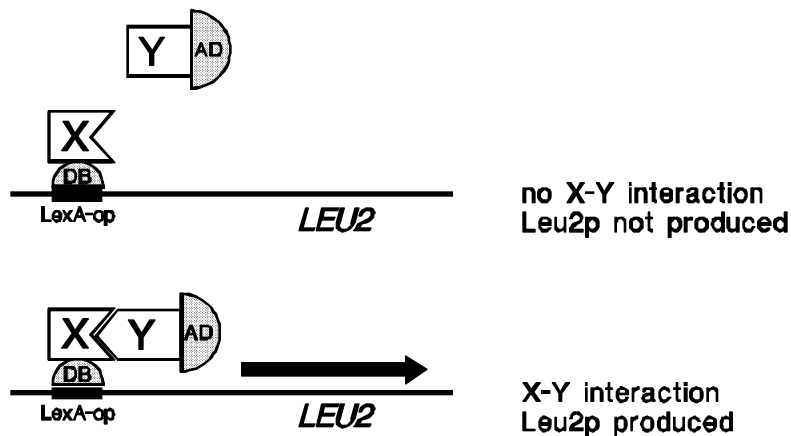


Figure 4 — Schematic of the yeast two-hybrid system. (A) The 'bait' protein, a fusion of the LexA DNA-binding domain to protein encoded by ORF X (LexA-DB/ORF X) is expressed constitutively in the presence or absence of galactose (\pm Gal). The 'prey' protein, a fusion of the B42 activation domain to protein encoded by ORF Y (B42-AD/ORF Y), is expressed only in the presence of galactose (+ Gal). (B) If proteins X and Y can interact, a functional transcription factor is formed and the *LEU2* reporter gene is expressed. Two-hybrid interactions are detected because they allow yeast to grow in a galactose-dependent fashion on medium lacking leucine.