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Wan-Sheng Lo
St. John's University

Diana C. Bartelt
St. John's University

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Abstract

We have constructed a plasmid library containing cDNAs from *Aspergillus nidulans* fused to the activation domain of yeast *GAL4*. When transformed into *S. cerevisiae* the Hf7c reporter host strain containing a plasmid with a "bait" DNA fused to the *GAL4* DNA binding domain, this library can be used to screen for interactions between the gene products encoded by the cDNA and the target protein encoded by the "bait" DNA.

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Wan-Sheng Lo and Diana C. Bartelt- Fungal Molecular Biology Group, Department of Biological Sciences, St. John's University, 8000 Utopia Parkway, Jamaica, NY 11439

We have constructed a plasmid library containing cDNAs from *Aspergillus nidulans* fused to the activation domain of yeast *GAL4*. When transformed into *S. cerevisiae* the Hf7c reporter host strain containing a plasmid with a "bait" DNA fused to the *GAL4* DNA binding domain, this library can be used to screen for interactions between the gene products encoded by the cDNA and the target protein encoded by the "bait" DNA.

The yeast two hybrid system (S. Fields and O-K Song, 1989 Nature **340**:245-247) is designed to detect protein:protein interactions and has been used successfully to identify substrates of several protein kinases. In an effort to identify protein substrates for the *Aspergillus nidulans* calmodulin-dependent multifunctional protein kinase [ACMPK; (Bartelt *et al.* 1988 Proc. Nat. Acad. Sci. *USA* **85**: 3279-3283)] we have constructed a cDNA library in a vector suitable for use in two-hybrid selection. Total RNA was prepared from R153 (wa3; *pyro*A4) mycelia harvested after 8, 12, 18 and 24 h of incubation at 37o C as described (Osmani *et al.*, 1987 J. Cell Biol. **104**: 1495-1504). Since Northern analysis of total RNA samples indicated that levels of mRNA encoding ACMPK were highest in samples collected after 8 h of incubation, polyA+ RNA was isolated from this sample using an mRNA Separator Kit (Clontech Laboratories, Inc., Palo Alto, CA). Synthesis of cDNA was accomplished using a c-Clone IITM cDNA Synthesis Kit (Clontech Laboratories, Inc.). First strand cDNA synthesis was primed with both oligo(dT)15 and a random d(N)6 primer to increase the representation of sequences in the 5' region of the cDNAs. Subsequent to second strand synthesis, the size of the cDNA products were analyzed by alkaline agarose gel electrophoresis and shown to range from 0.5 to 4.5 kb. The cDNA was protected with *Eco*RI methylase, digested with S1 nuclease to remove any hairpin loops, and single stranded ends were filled in using Klenow fragment, prior to the ligation of *Eco*RI linkers. The cDNA was digested with *Eco*RI and ligated into the multicloning site of pGAD424 (Bartel et al. 1993 in Cellular Interactions in Development: A Practical Approach Oxford University Press, pp.153-179), a plasmid containing the yeast *GAL*4 activation domain sequence and the yeast *LEU*2 and *E. coli ampr* genes. The resulting plasmid library was propagated in *E. coli* HB101 a strain which is auxotrophic for leucine. The cDNA library in *E. coli* HB101 (2.5×10^6 transformants/ ug DNA), will be made available through the Fungal Genetics Stock Center.

Plasmid library DNA suitable for two hybrid screening can be prepared from bacteria using kits such as a QIAfilter Maxi kit (QIAGEN, Inc., Catsworth, CA). To perform a two hybrid screen, "bait" DNA encoding a target gene product is cloned into pGBT9 (Figure 1A) containing *TRP1* and *ampr* genes (Bartel et al. 1993 *ibid*), such that the DNA was in frame with the sequence encoding the *GAL*4 DNA binding domain. The plasmid is used to transform the *S. cerevisiae* reporter host strain Hf7c [((*MAT***a** *ura*3-52 *his*3-200 *ade*2-101 *lys*2-801 *trp*1-901, *leu*2-3,112 *gal*4-542 *gal*80-538 *LYS*2::*GAL*1UAS- *GAL1*TATA-*HIS*3 *URA*3 :: *GAL*4 17mers (x3) - CyC*1*TATA-*lac*Z));

((Feiolotter, et al., 1994, Nuc. Acid Res. **22**:1502-1503))]. The *A. nidulans* cDNA library in pGAD424 (Figure 1B) is then transformed into the resulting strain as described (Gietz et al., 1992, Nucl. Acid Res. **20**:1425-1425). Cotransformants expressing *HIS3, LEU2* and *TRP1* are selected and screened for the expression of -galactosidase activity by a filter assay (Breeden. and Nasmyth, 1985, In Cold Spring Symposium on Quantitative Biology, **50**:643-650). The library is compatible with the MatchmakerTM Two Hybrid System (Clontech Laboratories, Inc.) which contains all of the components necessary to perform a two hybrid screen with the requisite positive and negative controls.

Figure 1. Restriction maps of the yeast shuttle vectors used in a two hybrid screen.

1A., plasmid pGBT9 containing a multicloning site (MCS) into which DNA encoding a target protein can be ligated such that it is in frame with the DNA binding domain of *GAL*4. The plasmid also contains the *TRP1* gene as a selectable marker in yeast and *ampr* gene for selection in bacteria. The vector is available as part of the MatchmakerTM Two Hybrid System from Clontech Laboratories, Inc. **1B.,** *A. nidulans* cDNA library in pGAD424, a plasmid containing the *LEU*2 gene as a selectable marker in yeast and *ampr* gene for selection in bacteria.

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