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L Hamer
Purdue University

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Abstract

With the purpose of examining interacting components of the septation process in *Aspergillus nidulans*, a yeast two-hybrid library of *A. nidulans* hyphal cDNA has been constructed. The yeast interaction trap is based on the in vivo detection of heterologous protein-protein interactions that will activate transcription in *Saccharomyces cerevisiae* (bakers yeast). Although the system potentially is powerful, much effort is necessary to identify background interactors due to heterologous complementation and non-specific initiation of transcription. A set of "false positives" was identified, which can be used to reduce the background in future yeast two-hybrid screens of *A. nidulans* libraries.

Knowing the hay from the needle: Yeast two-hybrid screens in Aspergillus nidulans - a set of clones to identify common "false positives" - L. Hamer, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907 USA

With the purpose of examining interacting components of the septation process in *Aspergillus nidulans*, a yeast two-hybrid library of *A. nidulans* hyphal cDNA has been constructed. The yeast interaction trap is based on the in vivo detection of heterologous protein-protein interactions that will activate transcription in *Saccharomyces cerevisiae* (bakers yeast). Although the system potentially is powerful, much effort is necessary to identify background interactors due to heterologous complementation and non-specific initiation of transcription. A set of "false positives" was identified, which can be used to reduce the background in future yeast two-hybrid screens of *A. nidulans* libraries.

Protein-protein interaction studies can be performed in the yeast two-hybrid system and several versions are available. This study pertains to the method of Gyuris *et al.* 1993 Cell 75:791-803. The system takes advantage of the composite nature of the GAL*4* transcription factor in that both an activation domain (AD) and a DNA binding domain (DBD) are necessary for transcription. The strategy employs the construction of a yeast strain containing a plasmid with the DBD fused to the (heterologous) protein for which interactors are searched (the bait). A cDNA library containing an AD fused to heterologous cDNA is introduced into the bait strain. When the cDNA interacts with the bait, transcription is initiated. The presence of the plasmids is selected by aminoacid prototrophy. Two reporter genes (LEU*2* and LacZ) are activated as a result of the interaction. To verify the specificity of the interaction the cDNA is typically introduced into other bait strains, in which an interaction is not expected.

Two-hybrid screens generally yield numerous "false positives" which, for a number of experimental situations, have been listed on the World Wide Web:
http://www.fccc.edu:80/research/labs/golemis/main_false.html
However, extensive two-hybrid screening for *A. nidulans* has not been reported, and the nature and frequency of "false positives" is unknown. In this paper several "false positives" that occurred repeatedly in a two-hybrid screen of an *A. nidulans* hyphal cDNA library are described.

The *A. nidulans* sepA gene is a member of the formin family and is involved in septation (Harris *et al.* 1997 EMBO J 16:4374-3483). The gene contains conserved motifs called FH1/2 domains. Several baits containing fragments of the gene have been constructed. Interaction between the FH1 domain and profilin has been reported in a number of the other formin family members (for review on the formin family members, see Frazier and Field 1997 Curr. Biol. 7:414-417). Likewise, as a positive control, interaction in the two-hybrid system was verified between the *A. nidulans* FH1 domain and profilin (L. Hamer, unpublished results). An FH2 domain-containing bait was used in a preliminary screen of A. nidulans hyphal cDNA library constructed in the AD-containing vector. The results are described below in Table 1.

Table 1. Results of test case: Identification of 56 putative interactors of the *A. nidulans sep*A FH2 domain. [a]

No. of clones	homologue
34	*Schizosaccharomyces pombe* ade3, tetrahydrofolate synthase
17	*A. niger* leu2, iso-propylmalate dehydrogenase
1	*Candida maltosa* spl1, tRNA splicing protein
4	other
Total 56	

[a]Interactors were selected on the basis of leucine prototrophy and §-galactosidase activity.

Tetrahydrofolate synthase (ade3, a *Schizosaccharomyces pombe* homologue) catalyzes three different reactions, some of which are involved in the biosynthesis of the aminoacids histidine and methionine (Genbank 2879878). leu2 is the iso-propylmalate dehydrogenase gene homologue of *A. niger* (Genbank S83228). spl1 encodes a *Candida maltosa* tRNA splicing protein homologue (Genbank 2492879). These three genes yield positive reactions either by simple complementation and/or by initiating non-specific transcription activation/overcoming splice defects. As a result, it was necessary to include a yeast DNA dot blot hybridization stop of initial positive clones using probes of these genes.

The cDNA library here described contains an insert size of 1-2 kbp. It seems likely that the use of libraries with smaller inserts could lower the risk for direct complementation of the auxotrophic markers. However small insert libraries may reduce the possibility of identifying clones with interaction domains distant from the 3' ends. Dividing the cDNA library into several subpools may also restrict the occurrence of "false positives" to single pools ra. _ r than the entire library. The "false positives" are deposited at FGSC as *E. coli* strains pLH105 (leu2), pLH107 (ade3), and pLH112 (*spl1*). The inserts can be released by EcoRI/XhoI restriction digestion.