1502–1503 Nucleic Acids Research, 1994, Vol. 22, No. 8

Construction of an improved host strain for two hybrid screening

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Received February 16, 1994; Revised and Accepted March 11, 1994

The two hybrid approach is a genetic screen for investigating protein–protein interactions (1). It has proven useful both for testing interaction between known proteins and for identifying previously unknown proteins which interact with a protein of interest. Several recent reviews have outlined the principles underlying this approach and have catalogued its successes (see 2 for example).

Using a two-hybrid technique involves the co-expression of two hybrid proteins in a yeast cell. Sequences coding for the first protein (the target) are fused in frame to those encoding the DNA-binding domain of the yeast *GAL4* transcriptional factor. Sequences coding for the second protein are fused to those encoding activation domain of *GAL4*. A functional GAL4 activator is created when the DNA binding and activation domains come together as a result of physical association between the two hybrids. *GAL4* activity is assayed using a reporter gene which depends on GAL4 for transcription (1). Though initially used to test for interactions between known proteins, the two hybrid approach has since been extended to large scale screens in which the target gene, fused to the DNA-binding domain of *GAL4*, is tested against a library of genomic or cDNA sequences fused to a transcriptional activation domain. Interacting proteins are selected by co-transformation of the target and the library fusions into an appropriate host yeast strain (3,4,5).

The host for such screens is the budding yeast, *Saccharomyces cerevisiae*. For use in two hybrid screening, host strains typically carry mutations which ensure that endogenous GAL4 is absent. In addition, *GAL80*, whose product normally inhibits GAL4 function, is mutated so as to avoid a requirement for galactose in the growth medium. The strains also carry auxotrophic markers, typically leucine and tryptophan, for selection of cells carrying the DNA-binding and activation domain vectors. Finally, these strains contain one or more reporter genes which are transcriptionally dependent on the reconstitution of functional GAL4.

A number of such strains have been constructed (see 2 for review). The earliest of these, GGY1::171 (1) depended solely on the transcription of a *lacZ* reporter gene to indicate physical interaction between two proteins. While useful, screening for β-galactosidase alone was too tedious for use in searching high-complexity libraries for interacting proteins. The addition of a second reporter gene in the form of a *GAL1*-driven *HIS3* gene increased the utility of the screen, since interacting clones could be identified by a growth selection on medium lacking histidine. Also, the use of two reporter genes had the added advantage of decreasing the background of false positives (2). Available versions of such host strains (e.g. YPB2 (2)) require the addition of the antimetabolite 3-amino-1,2,4-triazole (3-AT) (6) to the growth medium to increase the stringency of selection, since these strains are phenotypically prototrophic for histidine due to the use of a leaky *HIS3* alleles.

Our early experiments suggested that the use of 3-AT in the selective media had a number of disadvantages. First, it greatly

Table 1. Genotypes of strains

YPB3[1]	*MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 can^R gal4-542 gal80-538 LYS2::G-AL1$_{UAS}$-GAL1$_{TATA}$-HIS3*[3]
YM4136[2]	*MATα ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 gal80-538*
YPB2[1]	*MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 can^R gal4-542 gal80-538 LYS2::G-AL1$_{UAS}$-LEU2$_{TATA}$-HIS3 URA3::GAL4$_{17mers(\times 3)}$-CyC1$_{TATA}$-lacZ*
HF7c	*MATa ura3-52 his3-200 ade2-101 lys2-801 trp1-901 leu2-3,112 gal4-542 gal80-538 LYS2::GAL1$_{UAS}$-G-AL1$_{TATA}$-HIS3 URA3::GAL4$_{17mers(\times 3)}$-CyC1$_{TATA}$-lacZ*

[1]Gift from P.Bartel.
[2]Gift from M.Johnston.
[3]This plasmid contains the URA3 gene, making strains carrying it prototrophic for uracil.

Table 2. Testing of HF7c

Plasmid A[1]	Plasmid B[2]	His-[3]	blue[4]
pGBT95	–	–	–
–	pGAD-GH[6]	–	–
pGBT9	PGAD-GH	–	–
pCL1[7]	–	+	+
pGBT9-SNF1[8]	–	–	–
–	pGADGH-SNF4[8]	–	–
pGBT9-SNF1	pGADGH-SNF4	+	+

[1]Plasmids containing the DNA-binding domain of GAL4.
[2]Plasmids containing the activation domain of GAL4.
[3]Ability of transformants to grow on medium lacking histidine.
[4]Color of transformant following β-galactosidase assay, + blue, – white.
[5]ref. 2.
[6]ref. 7.
[7]This plasmid contains the GAL4 gene; ref. 1.
[8]G.H., this lab, unpub. data.

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increased the time required for growth selection of positive clones. Second, it restricted the subset of clones obtained in the two-hybrid screen. Therefore, we have developed a modified two-hybrid screening strain which we have called HF7c. This strain contains a GAL4-dependent HIS3 reporter gene which, due to its promoter, is not leaky and is, therefore, phenotypically auxotrophic for histidine. Thus HF7c does not require the use of the histidine antimetabolite. In addition, HF7c carries a *lacZ* reporter gene from YBP2. The first step in the construction of HF7c was the isolation of a strain identical to YPB3 except that it carried the MATα allele. This was accomplished by selecting progeny from a cross of parental strains YPB3 and YM4136 (see Table 1), which were prototrophic for uracil (indicating the presence of the *HIS3* construct integrated at the *LYS2* locus) and auxotrophic for histidine (indicating the presence of the *gal4-542* mutation). Outcrosses to tester MATa and MATα haploid strains allowed selection of the desired mating type. These progeny were then crossed to the strain YPB2 (see Table 1). The desired progeny would carry the *HIS3* allele from YPB2 and the *lacZ* gene from YPB2. The tighter *HIS3* allele was selected on the basis of lack of any growth on plates lacking histidine. The presence of the *lacZ* gene was confirmed by transformation of the *GAL4* gene into those strains displaying a tight *his*-phenotype. Those which gave a positive result in an assay for β-galactosidase activity were further characterized. HF7c was the one among these which gave the fastest growth, highest transformation efficiency and greatest β-galactosidase activity. HF7c is also auxotrophic for tryptophan and leucine, allowing selection of library and target plasmids. The complete genotype is given in Table 1.

We have subjected HF7c to the following tests to ensure that it conforms to all the requirements for use in two hybrid screens.
1) Either the *GAL4* gene or a control vector was introduced into HF7c, and transformants were tested for growth on media lacking histidine and β-galactosidase activity (Table 2).
2) HF7c was co-transformed with a pair of proteins previously shown to interact in the two hybrid system: the *SNF1* gene fused to the DNA-binding domain of *GAL4* and the *SNF4* gene fused to the *GAL4* activation domain. The transformants were selected by their ability to grow on medium lacking both tryptophan and leucine and were then tested for expression of both the *HIS3* and *lacZ* genes (Table 2).
3) To determine whether HF7c was suitable for large scale screening, we co-transformed the strain with the human *cdk2* gene fused to the DNA-binding domain of *GAL4* and a HeLa cDNA library fused to the activation domain. This same screen had been previously carried out in our laboratory using the host, YPB2, and genes encoding several interacting proteins had been identified (7). The same genes were among those identified using HF7c. However, because HF7c does not require the use of 3-AT, the time required from the start to the finish of such a screen was considerably less than with YPB2. Transformants were visible as small colonies as early as three days following transformation, whereas this took up to two week with YPB2.

HF7c allows a sensitive primary selection based on histidine expression, and a secondary selection based upon color detection. Criteria for the selection of those potential positives which should be characterized further has been discussed elsewhere (2, 8).

These assays indicate that HF7c is a suitable host for large scale screens using the two hybrid approach and should be useful to the many researchers currently using this technique to study protein–protein interactions.

ACKNOWLEDGEMENTS

The authors wish to thank P.Bartel and M.Johnston for their gifts of strains.

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