1778–1779 Nucleic Acids Research, 1994, Vol. 22, No. 9

A yeast mating-selection scheme for detection of protein–protein interactions

Christian Bendixen, Serge Gangloff[1] and Rodney Rothstein[1,*]
Department of Molecular Biology, University of Aarhus, C.F.Mollers Allé bldg 130, 8000 Aarhus C, Denmark and [1]Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, 701 West 168th Street, New York, NY 10032, USA

Received December 13, 1993; Revised and Accepted March 29, 1994

Recently, a new approach to the study of protein–protein interactions has appeared. The 'two-hybrid system' developed by Fields and coworkers (1, 2) can be used either to look for new interacting proteins, or to verify and characterize interactions between proteins that are likely to associate according to genetic or biochemical data. The two-hybrid system is a molecular genetic approach that takes advantage of the structural flexibility of the yeast transcription factor Gal4. The Gal4 protein contains two domains, the DNA-binding domain and the transcription activator domain. The two domains need not be part of the same protein to accomplish transcriptional activation (3). When the two domains are fused separately to two unrelated but interacting proteins, transcriptional activation can be achieved as a consequence of the protein–protein interaction.

Usually, the search for new interacting proteins using the two-hybrid system has been carried out by co-transforming a yeast strain containing an integrated copy of a UAS_{GAL1}-*lacZ* reporter gene with two plasmids (2, 4–6). One plasmid encodes a fusion of the DNA-binding domain of Gal4 to the protein of interest, while the other plasmid (the library plasmid) encodes a fusion of the transcriptional activation domain of Gal4 to randomly generated coding regions. Thus, the DNA-binding domain fusion will bind to the UAS_{GAL1} element upstream of the reporter gene. If a protein encoded by the library fusion plasmid interacts with the protein of interest, the transcription activation domain becomes co-localized upstream of the reporter gene, resulting in activation of transcription. Effective use of the two-hybrid system requires the generation of a large number of yeast transformants. Since transformation of yeast is still four orders of magnitude less efficient than that of bacteria, transformation can be the limiting step for an exhaustive screen of a cDNA library.

In this paper, we devise an easy method that eliminates the need for co-transformation and allows the user to search for interacting factors to an unlimited number of constructions with a *single* transformation. The transformants can be reused for additional searches via the mating procedures described below. Our strategy is to bring the two plasmids together in one cell by mating, rather than by transformation. Subsequently, diploids containing interacting fusion proteins are selected by using either a UAS_{GAL1}-*HIS3* or a UAS_{GAL1}-*LEU2* reporter construct that has been integrated into the yeast genome (4, 7). The specificity of the interaction is next confirmed by testing the ability of the selected clones to activate the expression of the *lacZ* reporter gene also integrated into the genome. As described in the original method, the elimination of false positives can also be facilitated by testing the recovered clones with an unrelated DNA-binding domain hybrid (8).

Two sets of strains were constructed for the assay, both contain the *lacZ* reporter gene and one of the two genes used for selection (Table 1). CBY12**a** and CBY12α differ only by their mating type and are used when selecting for expression of the UAS_{GAL1}-*LEU2* reporter construct. The corresponding pair, CBY14**a** and CBY14α, is used when selecting for the expression of UAS_{GAL1}-*HIS3*. Furthermore, all these strains are deleted for both *GAL4* and *GAL80*. The choice of strains depends on the selective markers on the plasmids encoding the Gal4 fusions. In many early studies, the DNA-binding fusion to the protein of interest is expressed from a plasmid carrying either *TRP1* (2, 5, 6), *LEU2* or *HIS3* (4) as the selective marker, while the library is constructed in plasmids containing *LEU2* (2, 5, 6) or *TRP1* (4). Thus, CBY12**a** and CBY12α are used when the plasmids carry *TRP1* and *HIS3*, and CBY14**a** and CBY14α are used when the plasmids contain *TRP1* and *LEU2*.

We used this method to isolate protein domains that interact with a sequenced open reading frame that we have been studying in the laboratory (S.G. and R.R., unpublished observations). The ORF was fused in-frame with the *GAL4* DNA-binding domain

Table 1. Genotypes of the strains used in this study

Strain	Genotype
CBY12**a**	*MAT***a** *ade2 his3 leu2*::UAS_{GAL1}-*LEU2 trp1 URA3*::UAS_{GAL1}-*lacZ gal4*Δ *gal80*Δ
CBY12α	*MAT*α *ade2 his3 leu2*::UAS_{GAL1}-*LEU2 trp1 URA3*::UAS_{GAL1}-*lacZ gal4*Δ *gal80*Δ
CBY14**a**	*MAT***a** *ade2 his3 leu2 trp1 URA3*::UAS_{GAL1}-*lacZ gal4*Δ *gal80*Δ *LYS2*::UAS_{GAL1}-*HIS3*
CBY14α	*MAT*α *ade2 his3 leu2 trp1 URA3*::UAS_{GAL1}-*lacZ gal4*Δ *gal80*Δ *LYS2*::UAS_{GAL1}-*HIS3*

* To whom correspondence should be addressed

on plasmid pGBT9 containing *TRP1* for selection (2) to create pWJ455 and was transformed into CBY14**a**. Three pools of a plasmid library, marked with *LEU2* and containing random yeast DNA fused to the transcription-activation domain of Gal4 (2) were transformed into the CBY14α strain. Approximately 50,000−100,000 transformants from each pool were collected from the plates in 10 ml of YPD, and 0.5 ml aliquots were stored in 15% glycerol at −80°C.

After thawing, 500 μl of each of the three pools of *MAT*α transformants were next plated at a density of $0.3-1\times10^5$ cells per plate (85 mm) onto 10 plates of synthetic complete medium lacking leucine to select for the library plasmid. At this density, the cells grow as distinct colonies that almost form a 'lawn'. The *MAT***a** strain is plated as a lawn ($>1\times10^6$ cells per plate) on 30 synthetic complete plates lacking tryptophan, which selects for the plasmid encoding the DNA-binding fusion. After 2−3 days at 30°C, the two strains are mated by replica-plating one *MAT***a** lawn and one *MAT*α lawn onto a single YPD plate (for information on media and yeast handling, please refer to reference (9)). Cells of opposite mating type fuse to form diploids on YPD. After one day at 30°C, the efficiency of mating is tested by replica-plating to medium selecting for the diploids (in this case SC-trp-leu) and varies from 20 to 80%. In addition, the plates are replica-plated to medium that lacks tryptophan, leucine and histidine. This selects directly for diploids harboring a cDNA-$Gal4_{activation\ domain}$ fusion that can activate transcription of the reporter gene. When *HIS3* is the reporter gene, its expression in the absence of an activator is modulated by using a histidine competitor (3-amino-1,2,4-triazole) at a final concentration of 25 mM (10).

In our experiment, between 50−100 colonies grew on each of the triple-dropout plates. The colonies were directly transferred to nitrocellulose filters and re-screened for β-galactosidase activity using standard procedures (2, 4). Since the yeast activation domain library we used was constructed with DNA from a *GAL4* containing strain, we performed control experiments with wild-type Gal4 to determine the kinetics of blue color appearance. We found that the blue color appears in less than one hour of incubation at 37°C. Therefore, in our cloning experiment, approximately 80% of the transformants were discarded since they displayed blue color before one hour. This is not a problem with libraries constructed with DNA other than *S.cerevisiae*. Approximately 20% of the clones (~300), which were capable of activating the *HIS3* reporter gene, failed to activate the β-galactosidase reporter. These may represent protein domains specifically affecting *HIS3* expression, and were also discarded. Eight library plasmids were recovered that gave a blue signal after 4 hours of incubation. PCR analysis with primers specific for the intact *GAL4* gene was performed to test whether any of these eight clones contain the *GAL4* activation domain. Three contained *GAL4* DNA. Restriction analysis of the five other clones indicated that four of them were identical, the fifth exhibiting an identical restriction pattern but with 3 kb of additional DNA. Amongst these 5 clones, we found a common 1.5 kb fragment with a 1.4 kb ORF that interacts specifically with pWJ455 (S.Gangloff, J.McDonald, L.Arthur, C.Bendixen and R.Rothstein manuscript in preparation).

The mating-selection scheme described here has a number of advantages compared to previous protocols that involve co-transformation followed by screening. Because the cells can be stored indefinitely when frozen, transformation of the library only has to be performed once. The cells are then streaked fresh from the frozen pools for every new screen. The direct selection for interactions is very useful because fewer plates are needed but also because the number of colonies per plates is not as critical for selection as it is for screening. The use of two reporter genes, one for selection and one for screening, has the further advantage that it lowers the likelihood of getting false positives. Such false positives, which could arise either from mutation or from cryptic DNA-binding of the cDNA-$Gal4_{activation\ domain}$ fusion to sequences other than Gal4-binding sites, are less likely to cause non-specific activation of more than one of the reporter genes (8). A potential disadvantage of this mating-selection scheme is the increased risk of creating a bias in the representation of the library plasmids by the transformation and growth of the library in yeast, prior to mating.

ACKNOWLEDGEMENTS

We thank Stan Fields, Pierre Chevray and Ganjam Kalpana for sharing reagents. We also thank Stan Fields and Steve Sturley for critical reading of the manuscript. This work was supported by a grant from The Danish Medical Research Council to C.B. and by NIH grants and an NSF grant to R.R.

REFERENCES

1. Fields,S. and Song,O. (1989) *Nature* **340**, 245−246.
2. Chien,C.T., Bartel,P.L., Sternglanz,R. and Fields,S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9578−9582.
3. Ma,J. and Ptashne,M. (1987) *Cell* **48**, 847−853.
4. Chevray,P.M. and Nathans,D. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5789−5793.
5. Hardy,C.F., Sussel,L. and Shore,D. (1992) *Genes Dev.* **6**, 801−814.
6. Luban,J., Bossolt,K.L., Franke,E.K., Kalpana,G.V. and Goff,S.P. (1993) *Cell* **73**, 1067−1078.
7. Ronne,H. and Rothstein,R., unpublished result.
8. Bartel,P., Chien,C.T., Sternglanz,R. and Fields,S. (1993) *BioTechniques* **14**, 920−924.
9. Sherman,F., Fink,G.R. and Hicks,J.B. (1986) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
10. Durfee,T., Becherer,K., Chen,P.L., Yeh,S.H., Yang,Y., Kilburn,A.E., Lee,W.H. and Elledge,S.J. (1993) *Genes Dev.* **7**, 555−569.