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Detection of protein–protein interactions using different vectors in the two-hybrid system

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The yeast two hybrid system originally developed by Fields and Song is a powerful *in vivo* assay for protein–protein interactions (1,2). In its initial version, it was based on the co-expression of two proteins fused to transcriptional activator and DNA-binding domains of the yeast protein GAL4, respectively. If a physical interaction occurs between the two proteins, it brings the GAL4 domains in sufficient proximity to activate the GAL4-dependent transcription of a reporter gene (such as *lacZ*).

The initial pair of vectors (pGAD2F/pMA424; for clarity, pairs of vectors will always be cited in the order GAL4-activation/GAL4-DNA binding domain) have been successfully used in several studies (3,4). However, they are large plasmids (>13 kb) with few available cloning sites. More manageable second-generation pairs of vectors have now been developed which allow both easier constructions and screening of libraries to search for proteins interacting with the protein of interest. Some of these pairs, such as pGAD424/pGBT9 (5) have been designed for the expression of relatively low amounts of fusion transcripts from a truncated version of the ADH promoter. Although low production of fusion proteins may be advantageous to overcome their possible toxic effect, it may also affect the sensitivity of the interaction assay. To study this, we have analyzed the sensitivity of the two hybrid assay for several proteins, using a combination of commonly available GAL4-based vectors. Our study includes two sets of second generation vectors, namely pGAD424/pGBT9 (5) and pAS2/pACTII (6,7; S.Elledge, personal communication) as well as the original pair, pGAD2F/pMA424 (1,2). We assayed yeast nuclear proteins (PRP splicing factors) as well as viral proteins (from the human hepatitis B virus and the human T leukemia virus type 1) for heterodimer or homodimer formation.

All these 2-micron vectors contain the promoter region and the terminator of transcription of the ADH gene which direct expression of the fusion proteins. However, the promoter regions in the second generation vectors are shorter and the relative position of the various elements in the plasmids is different. Results of two hybrid assays performed with various combinations of these vectors are presented in the table. For six out of seven interactions, sensitivity of the assay was significantly reduced when pGBT9 was used instead of pMA424, with a 5 fold reduction of the maximal stimulation (PRP9/PRP21 interaction). An even more dramatic drop in sensitivity (5–50 fold) was observed when pGAD424 was used instead of pGAD2F. For 5 different interactions, the assay with the pGAD424/pGBT9 set of vectors was roughly 50 to 100 time less sensitive than with the original vectors (pGAD2F/pMA424). Therefore, an interaction may sometimes escape detection when assayed with the pGAD424/PGBT9 set, since activities below 0.5 units are not detected in a qualitative assay on colonies (PRP9/PRP21, for example).

However, it is noticeable that in one case (core/core), the detection of interaction was more modestly affected (less than a ten-fold decrease in β-galactosidase units) and in the last case, *tax/tax*, it was not significantly changed. These observations suggest that the limiting parameter for the detection of interactions in the two-hybrid system may differ from one interaction to another. It could be that similar steady-state amounts of proteins are achieved with different transcriptional rates for proteins that are highly stable in yeast. In this regard, we note that a dramatic effect of the vector combination was observed only for the homologous yeast nuclear proteins.

Two very recently developed vectors (pACTII and pAS2) allow a sensitive measurement of interactions. This result should be underlined, since pACTII has a short ADH promoter similar to those of pGAD424 and pGBT9 plasmids. Therefore, the sensitivity of the assay did not simply correlate with the size of the fragment of the ADH promoter driving the expression of the fusion transcript. A possible important parameter could be the relative position of the various cassettes in the different vectors.

A toxic effect of some fusion proteins produced from the original two-hybrid vectors has been reported (3). Such a toxic effect, also observed with the PRP11 protein, was much less severe when the protein was produced from the pGAD424 vector (data not shown). Therefore, experimental strategies should be carefully designed: on the one hand, overproduction of a toxic protein may lead to the appearance of fast-growing recombinant colonies (large white colonies among small blue ones in a *lacZ* reporter detection assay on plates); on the other hand, low production of fusion proteins may preclude the detection of some protein–protein interactions. There is no reason to suspect that weak interactions have less biological significance than strong ones (7). Our results suggest that certain combinations of vectors may introduce a bias because weak interactions may be overlooked. This fact should be kept in mind when screening

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Table. Protein–protein interactions measured in two-hybrid assays using different vectors

Activation domain/ DNA binding domain	pGAD2F/ pMA424	pGAD2F/ pGBT9	pGAD424/ pMA424	pGAD424/ pGBT9	pACTII/ pAS2
PRP9/PRP9[1]	100[4]	25	1.5	0.8	40
PRP9/PRP21	25	5	1	0.25	25
PRP21/PRP9	40	10	1.5	0.8	57
PRP11/PRP21	35	20	1	0.6	37
PRP21/PRP11	60	15	1.5	0.8	48
core/core[2]	332	221	66	40	N.D.
Tax/Tax[3]	42	43	43	40	N.D.

[1]PRP fusion proteins expressed from the pGAD2F and pMA424 vectors have been previously described (3,4).
[2]The core protein of human hepatitis B virus (8).
[3]The tax protein of the human T leukemia virus type I (9).
[4]Y526 yeast cells were transformed and β-galactosidase assays were performed as previously described (3). Background levels are around 0.05 units. The combinations of any of these fusion proteins with an irrelevant partner gave activities below 1 unit with the pGAD2F/pMA424 set. Numbers are means of duplicates made on three independent transformants and experimental errors among duplicates were within a 10% range.

libraries, if an exhaustive identification of proteins interacting with a protein of interest is desirable. For the analysis of interactions between known proteins it might be useful to assay several combinations of the vectors available.

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REFERENCES

1. Fields,S. and Song,O.K. (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. Legrain,P., Chapon,C. and Galisson,F. (1993) *Genes Dev.* **7**, 1390–1399.
4. Legrain,P. and Chapon,C. (1993) *Science* **262**, 108–110.
5. Bartel,P.L., Chien,C.-T., Sternglanz,R. and Fields,S. (1993) in Hartley,D.A. (ed.), *Using the two-hybrid system to detect protein–protein interactions*. Oxford University Press, Oxford, pp. 153–179.
6. Durfee,T., Becherer,K., Chen,P.-L., Yeh,S.-H., Yang,Y., Kilburn,A., Lee,W.-H. and Elledge,S.J. (1993) *Genes Dev.* **7**, 555–569.
7. Harper,J.W., Adami,G.R., Wei,N., Keyomarsi,K. and Elledge,S.J. (1993) *Cell* **75**, 805–816.
8. Tiollais,P., Pourcel,C. and Dejean,A. (1985) *Nature* **317**, 489–495.
9. Lee,T.-H., Coligan,J.E., Sodroski,J.G., Haseltine,W.A., Salahuddin,S.Z., Wong-Staal,F., Gallo,R.C. and Essex,M. (1984) *Science* **226**, 57–61.