Benchmarks

Use of the Two-Hybrid System and Random Sonicated DNA to Identify the Interaction Domain of a Protein

BioTechniques 21:430-432 (September 1996)

We have previously used genetic selection from random sonicated DNA fragments in eukaryotes as a means of identifying DNA segments with regulatory functions (9,13,14). Here we combine this approach using sonicated DNA with the yeast two-hybrid system (6) to delineate DNA segments that encode protein-protein interaction domains. As a model, we chose the interaction of the simian virus 40 (SV40) large T antigen with the cellular control/tumor suppressor protein p53 (5). The interaction assay in yeast is described in Figure 1.

One hundred micrograms of plasmid pCMVTag (12) carrying the genomic DNA sequence of the SV40 large T antigen was sonicated using the Branson sonifier system (Branson Ultrasonics, Danbury, CT, USA). Sonicated fragments of approximately 600–1400 bp were repaired to blunt ends by treatment with T4 and Klenow polymerases and then phosphorylated with T4 kinase.

A fragment library was made by ligating the sonicated DNA fragments into the *Sma*I site of linearized pGAD plasmid (1) in a molar ratio of 45:1 (fragments:vector). After the repair reaction and ligation, the DNA was extracted with phenol and precipitated with isopropanol.

Yeast strain Y153 carrying *HIS3* and *lacZ* reporter genes (4) was transformed to Trp prototrophy with pVA3 that contained murine p53 in pGBT9 (11). A single colony was grown in minimal medium lacking tryptophan and transformed with an in vitro-generated library of random sonicated fragments by the modified lithium acetate method (7). Transformants were selected on minimal medium lacking leucine, tryptophan and histidine, but including 30 mM 3-aminotriazole (3-AT), and incubated at 30°C for 3–5 days. The addition of 3-AT was necessary to inhibit

growth of false-positive clones resulting from basal transcription of the *HIS3* gene in the absence of any *GAL4* function. Of approximately 2300 transformants, three were able to grow on the selection plates. The transformants were then assayed for β -galactosidase activity using a filter assay (2). Of these three His⁺ colonies, two turned blue in the presence of the chromogenic substrate X-gal (5-bromo-4-chloro-3-indolyl- β -D-galactoside).

pGAD-based plasmids encoding the candidate interacting domain of the large T antigen were isolated as described (10). The cloned genomic inserts of the T antigen were amplified by polymerase chain reaction (PCR) using

primers corresponding to regions of the vector flanking the cloning site. Both insert DNAs had a size of approximately 1200 bp.

After purification on a preparative agarose gel, the amplified fragments were directly sequenced and compared to the known DNA sequence of the large T antigen (Figure 2). Both sequences encoded a portion of T antigen joined in-frame with the GAL4 activation domain. One of the positive clones turned dark blue in the filter test and contained the T antigen segment encoding amino acids 238 to 632, which corresponded to the previously defined interaction domain (amino acids 259 to 517) (5), while the other stained light

Figure 1. Selection of a protein-protein interaction domain by the interaction assay in yeast.

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Figure 2. Two different fragments of SV40 large T antigen selected for interaction with murine p53. The known region of SV40 large T antigen sufficient for binding to p53 cellular control/tumor suppressor protein (5) is depicted as a shaded box. Below are the two interacting segments selected from a library of sonicated fragments from a 7743-bp plasmid pCMVTag (12) harboring the genomic DNA sequence of the SV40 large T antigen.

blue in the filter test and contained amino acids 307 to 698 (Figure 2).

In summary, we have developed a method combining the use of random sonicated DNA and the yeast two-hybrid system in order to identify protein interaction domains. The advantages of the described method are as follows: First, it allows for randomized fragmentation of the gene of interest. Second, all manipulations are well established, resulting in a substantial saving of time. Alternative DNA fragmentation procedures are also conceivable, such as complete or partial restriction digestion or partial digestion DNase I in the presence of manganese, which generates double-stranded, slightly staggered DNA breaks (3). We have also successfully used this method to identify the interaction domain of a mammalian protein that binds to the C-terminal domain (CTD) of RNA polymerase II (unpublished). Once narrowed to a defined region, protein-protein interaction can be further dissected genetically by PCR mutagenesis in yeast (8).

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We thank Pamela Mitchell, Joy Alcedo and John Silke for critical reading of the manuscript. This work was supported by the Kanton Zürich and the Schweizerischer Nationalfonds. Address correspondence to Walter Schaffner, Institut Für Molekularbiologie, Universität Zürich, Winterthurerstr.190, CH-8057 Zürich, Switzerland.

Received 14 November 1995; accepted 26 February 1996.

Igor Stagljär, Jean-Pierre Bourquin and Walter Schaffner
*Universität Zürich
Zürich, Switzerland*