Benchmarks

Conversion of Pipet-Tip Boxes into Secure Storage Containers for Microcentrifuge Tubes

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We describe an inexpensive and convenient container that solves some common problems associated with storing microcentrifuge tubes in refrigerators and freezers. It is made from a plastic box that comes prepackaged with pipet tips, and we have used FISHERbrand® Redi-Tip boxes Catalog No. 21-197-8H (size 200 μ L) or Catalog No. 21-197-2E (size 0.5–10 μ L) (Fisher Scientific, Pittsburgh, PA, USA) with equal success. When complete, each container will hold 24 microcentrifuge tubes. The unit has an attached lid, which snaps shut so that the enclosed tubes will not dislodge from their original positions when the unit is turned upside down. Furthermore, the container can be labeled on all sides for easy identification, its contents can be clearly viewed through the transparent lid and multiple units can be neatly stacked. By using simple tools, a container can be manufactured in minutes from materials that would normally be discarded. The operation requires a metal core borer with a diameter slightly larger than that of the tubes to be stored (we use a 12-mm-diameter cork borer), a heat source such as a Bunsen burner, a pair of tweezers to remove excised pieces of plastic and a hood in which to melt the plastic since this can generate some toxic fumes.

The procedure is simply to excise the appropriately sized pieces of plastic in the inner white support rack at regularly spaced intervals by applying the heated end of the metal cork borer to the plastic rack. Starting at the upper left-hand corner of the rack and having the cork borer heated to a red-hot stage, we push the heated tool quickly into the support, centering the punch within the first 4 holes in the rack. Then we use tweezers to pull the plastic from the cork borer, reheat if necessary and punch the next hole. Repeating this procedure, 4 rows of 6 holes for 1.5-mL or 2.0-mL tubes can be produced in a

few minutes. For 0.5-mL tubes, either melt smaller holes with a smaller diameter borer or convert larger holes by covering them with a piece of tape and cutting an "X" into the tape to support the smaller tubes.

By producing these secure and adaptable containers, we recycle plastics that would otherwise get thrown into the landfill, and we optimize freezer and refrigerator storage space in a very cost-effective manner.

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Screening for Recombinant Plasmids in Yeast Colonies of the Two-Hybrid System Using PCR

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The two-hybrid system (2,5) is an ingenious tool to detect protein-protein interactions. A number of gene products could be confirmed to be actual interactors (1,7,8), where others were identified by using cDNA libraries (4,6,9). For any purpose it is necessary to clone the genes/gene repertoires of the "query" protein and its putative partner(s) in two special plasmids, pGAD424 and pGBT9, respectively. Plasmids with correct inserts are transferred into yeast cells (strain HF7c), selected for several days on appropriate plates and then assayed for reporter

Figure 1. Demonstration of PCR of a selected yeast colony after directly lysing the cells and amplifying the plasmid DNA. PCR was routinely performed for 60 cycles, cycle program: 30 s at 94°C, 30 s at 54°C and 30 s at 72°C plus 1-second per cycle. The products were analyzed by electrophoresis in 1% agarose/ethidium bromide. The following primers were used: A: 5'-CGCGT-TTGAATCACTACAGG-3' (primer for vector pGAD424), B: 5'-GCGACATCATCATCGGAAGA-3' (primer for vector pGBT9) and C: 5'-GC-ATGCCGGTAGAGGTGT-3' (common back-primer). Lane 1 shows the reaction product using primers B and C, lane 2 shows the reaction products using primers A and C and lane 3 shows the reaction products using primers A, B and C. Lane 4 contains the 1-kb ladder (Life Technologies, Gaithersburg, MD, USA).

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gene activation. Once having positive candidates, the subsequent task is to identify among them the genes of proper ligands to the “query” protein.

The common approach to characterize recombinant plasmids from bacterial clones is poorly applicable to the situation in yeast because the DNA copy number is generally too small for direct visualization on agarose gels. To get sufficient quantities for handling and analysis (sequencing, restriction mapping, etc.), candidate colonies have (i) to be grown in liquid culture for at least one day. Vectors from them are (ii) usually retransferred into *E. coli* by electroporation for subsequent insert analysis. This procedure is not only time-consuming, but it may lead to undesired plasmid alterations. The commercial supplier of the MATCHMAKER™ Two-Hybrid System (CLONTECH Laboratories, Palo Alto, CA, USA) explicitly stresses the segregation prob-

lem and therefore recommends re-assaying of multiplied plasmids for reporter gene activity in each case. According to our observations, even in the case of small inserts, growth in liquid culture often led to loss of inserts. This results in false-negative experiments and an unsatisfactory reproducibility of the results. To overcome these difficulties, our method of choice is to analyze plasmid inserts directly after primary selection of yeast colonies by polymerase chain reaction (PCR). Direct amplification has been described for bacteria (3) and for genomic DNA from yeast cells as well (11). This method circumvents the need to prepare overnight cultures of yeast and the isolation step by directly lysing selected bacterial colonies in an appropriate PCR mixture.

To apply this method to the special MATCHMAKER plasmids in yeast cells, the following experiments were

performed: A liquid culture of yeast strain HF7c (the strain supplied in the MATCHMAKER Two-Hybrid System kit [CLONTECH]) was transformed with pGBT9 + “query”-insert and pGAD424 + repertoire-insert by means of electroporation (10) and cultured on plates in media devoid of leucine, tryptophan and histidine, but containing 16 nM aminotriazol. After an incubation for 3 days at 30°C, the first colonies can be picked for color assay and PCR.

For the latter, a pinch of each colony was added to 50 µL of a PCR mixture preheated to 94°C containing the following components: 0.2 mM of each dNTP, 2.5 mM MgCl₂, 16 mM (NH₄)₂SO₄, 50 mM Tris-HCl (pH 8.8), 0.01% Tween-20, two units of Combi-Pol-Polymerase (InViTek GmbH, Berlin-Buch, Germany) and 500 nM of each primer. Two distinct upstream primers (with different distances to the multicloning site) and one common

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Figure 2. Maps of the used plasmids A) pGAD424 and B) pGBT9 as described in the MATCHMAKER Two-Hybrid System. The primer sites are indicated with arrows: A is within the GAL4 activation domain, B is at the end of the GAL4 binding domain and C is in the transcription termination region including the *Sph*I-site. The length of the PCR products amplified with primers A, B and C and empty plasmids as templates are about 700 bp for combination A/C and 250 for B/C.

backprimer were synthesized and used for PCR (for sequences see Figure 1). The primer pairs were used either separately or simultaneously. In that way, it is possible to screen for both plasmids in one reaction according to the distinguishable distances of the primer pairs (Figure 2). After adding a yeast colony to the PCR mixture, the sample was vigorously stirred and kept at 94°C. Visible cloudiness did not disturb the reaction. The PCR cycles were started as specified in Figure 1. As shown in Figure 1, clear-cut reaction products could be demonstrated using this procedure. The products were cleaned and sequenced by using primer C (Figure 1) as sequencing primer (which is 82 bp above the insertion site) or the sequencing primer supplied with the system with unequivocal results.

These results indicate that the "colony-screening-PCR" is highly reliable, saves time and is applicable to partner gene search experiments by the aid of the yeast two-hybrid system. It avoids long-time liquid culturing and intermediate hosts and thus the danger of spontaneous segregation events. The method fixes the DNA status at the moment of the biological assay. The primers and reaction conditions are applicable for all amplifications, indepen-

dent of whether one or both plasmids (pGAD424 and pGBT9) are involved.

REFERENCES

1. Aelst, L.V., M. Barr, S. Marcus, A. Polverino and M. Wigler. 1993. Complex formation between RAS and RAF and other protein kinases. *Proc. Natl. Acad. Sci. USA* 90:6213-6217.
2. Chien, C.T., P.L. Bartel, R. Sternglanz and S. Fields. 1991. The two-hybrid system: A method to identify and clone genes for proteins that interact with a protein of interest. *Proc. Natl. Acad. Sci. USA* 88:9578-9582.
3. Costa, G.L. and M.P. Weiner. 1994. Screen-test recombinant screening in one day. *Strategies* 7:35-37.
4. Durfee, T., K. Becherer, P.L. Chen, S.H. Yeh, Y. Yang, A.E. Kilburn, W.H. Lee and S.J. Elledge. 1993. The retinoblastoma protein associates with the protein phosphatase type 1 catalytic subunit. *Genes Dev.* 7:555-569.
5. Fields, S. and O. Song. 1989. A novel genetic system to detect protein-protein interactions. *Nature* 340:245-247.
6. Hardy, C.F.J., L. Sussel and D. Shore. 1992. A RAP-1 interaction protein involved in transcriptional silencing and telomere length regulation. *Genes Dev.* 6:801-814.
7. Kato, G., W.M.F. Lee, L. Chen and C.V. Dang. 1992. Max: functional domains and interaction with c-Myc. *Genes Dev.* 6:81-92.
8. Li, B. and S. Fields. 1993. Identification of mutations in p53 that affect its binding to SV40 T antigen by using the yeast two-hybrid system. *FASEB J.* 7:957-963.
9. Luban, J., K.L. Bossolt, E.K. Franke, G.V. Kalpana and S.P. Goff. 1993. HIV type 1 Gag protein binds to cyclophilins A and B. *Cell* 73:1067-1078.
10. Potter, H. 1993. Application of electroporation in recombinant DNA technology. *Methods Enzymol.* 217:461-483.
11. Sathe, G.M., S. O'Brien, M.M. McLaughlin, F. Watson and G.P. Livi. 1991. Use of polymerase chain reaction for rapid detection of gene insertions in whole yeast cells. *Nucleic Acids Res.* 19:4775.

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