


The work was supported by BBSRC. Karim Roder was supported by an EU Biotechnology training fellowship (BIO-CT-94-7584). The yeast strain yWAM2, the plasmids pPC86, pRS315HIS, Y11 and 59.7 were kindly provided by Randall R. Reed (Howard Hughes Medical Institute Research Laboratories, The Johns Hopkins University School of Medicine, Baltimore, MD, 21205-2185, USA). Address correspondence to Michael Schweizer, Genetics and Microbiology Department, Institute of Food Research, Norwich Research Park, Colney, Norwich, NR4 7UA, UK. Internet: michael.schweizer@bbsrc.ac.uk

Received 19 June 1995; accepted 13 November 1995.

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Device to Facilitate Colony Hybridization


Identification of specific genomic and cDNA clones propagated in bacteria is generally accomplished by transferring the bacterial colonies onto nylon or nitrocellulose filters, followed by hybridizing the filters with labeled probes (3,5). A critical step in this procedure is lysis of the bacterial cells and denaturation of the DNA on the filters. Commonly, this is performed by wetting the filters in a denaturing solution consisting of 0.5 N NaOH/1.5 M NaCl, followed by neutralization with a solution consisting of 1 M Tris-HCl, pH 7.4/1.5 M NaCl (5). During these steps, adequate care has to be taken to ensure that the duration of denaturation and neutralization of each of the filters is kept uniform. In addition, since each filter is transferred between solutions, care should be taken to prevent cross contamination of the colonies by the solutions getting on the top surface of the filters. This procedure, although effective, can be cumbersome and time-consuming, particularly when dealing with a large number of filters. Identification of false positives due to cross contamination can be a problem, particularly when using high-density colony filters. Alternative methods using a microwave oven to lyse cells and denature the DNA on filters have also been used—however, only with nylon filters (1). In addition, the duration of the treatment and the limited number of filters that can be processed at a time does not significantly decrease the time expended.

We describe the use of a simple and inexpensive device to facilitate colony lysis. This device, shown in Figure 1, consists of a support constructed from closely meshed fiberglass netting (window screen available at any hardware supply store) held in place by a frame made of Delrin® plastic (Delrin 500 series thermoplastic; Du Pont, Wilmington, DE, USA). Filters (Hybond™-N nylon filters; Amersham, Arlington
Heights, IL, USA), generated by either arraying the clones using a robotic work station (2,4) or by colony lifts (5), are placed on the netting wetted with water; and the entire assembly is transferred to a larger tray (CESCO-LITE photo tray; Photo-quip, Fernandina Beach, FL, USA) containing a predetermined volume (25 mL in this case) of the denaturing solution in the center of the tray. Upon contact with the denaturing solution, the liquid spreads outward from the center of the netting, thereby wetting all the filters uniformly. Following incubation for an appropriate length of time (5 min in this case), the entire assembly of the filters and the netting is transferred to a second tray containing the neutralizing solution. The filters are allowed to air-dry on the netting by placing the assembly on a sheet of filter paper (3MM chromatography paper; Whatman International, Maidstone, Kent, England, UK) prior to cross-linking the DNA using ultraviolet light. The filters can be either screened by hybridization or stored for later use.

There are several advantages in using this device compared with the conventional method of manually transferring the filters using forceps. Since the filters are initially overlaid on the netting prior to soaking with the denaturing solution, all the filters are wetted uniformly with no variation in the duration of treatment, thereby producing consistent results. The netting support allows for use of small solution volumes, which, in turn, prevents the liquid from passing through the netting and, instead, directs the liquid to the periphery, essentially eliminating any cross contamination. The transfer of the filters to the neutralizing solution by moving the entire assembly of filters and netting greatly reduces time expenditure and virtually eliminates any damage to the filters due to manual manipulation. In our hands, a netting

Figure 2. Hybridization of filters processed using the support device. A) High-density filter (384-well, 5 x 5 array) arrayed with bacterial colonies containing pUC plasmid probed with radiolabeled pUC plasmid DNA. B) Colony lift of bacterial colonies containing plasmid clones of human genomic DNA probed with a radiolabeled oligonucleotide specific for Alu repeated DNA.
assembly with dimensions of 14 × 17 in. can accommodate 12 high-density arrayed filters (8 × 12 cm) or 15 circular filters (82 mm diameter). The overall results produce a significant increase in the throughput of the number of filters that can be processed with consistent results and the absence of any cross contamination. Cleaning the netting assembly is easily accomplished by rinsing with water and air drying. Compressed air can be used for drying as an alternative to air drying.

We have successfully used this device with consistent results for the analysis of high-density arrayed filters or colony lifts using filter circles (Figure 2). The vast amount of information being generated as a result of the advances in the human genome project necessitates rapid methods to facilitate the analysis of a large number of clones. This device can be easily adapted to an automated workstation designed to carry out the processing of a large number of filters. In summary, this simple and inexpensive device should be a valuable tool to aid in the rapid analysis of specific clones in the number of laboratories engaged in genome research.

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This work was performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under Contract No. W-7405-ENG-48. Address correspondence to Santosh S. Arcot, Human Genome Center, L-452, Biology and Biotechnology Research Program, Lawrence Livermore National Laboratory, Livermore, CA 94551, USA. Internet: arcot1@llnl.gov

Received 5 September 1995; accepted 26 October 1995.

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Practical Method for Cloning cDNAs Generated in an mRNA Differential Display


The technique of differential display of messenger RNA species, originally described by Liang and Pardee (7), is a powerful tool for cloning genes that are differentially expressed in various eukaryotic cells and organs or under altered conditions. This technique is sensitive and reproducible, and it is faster than either differential screening of a cDNA library or subtractive hybridization (3,7). This method has been used to identify and clone genes from mammalian cells (1,5-7), as well as plant tissues (8,11,12). Although most of the differentially displayed bands on a gel do represent specific mRNAs, it is usually difficult to clone the cDNAs representing appropriate mRNA (2,4). This is mainly due to the contamination of specific DNA bands by heterogeneous sequences. The major sources of contamination are DNA present in the total RNA used for cDNA synthesis, presence of undetectable overlapping bands along with unique bands in display gels and copurification of neighboring bands. It has been often found that each band recovered from the display gel contains three or more different sequences (2,4). The ratio of nonspecific cDNA will be greatly increased during the lengthy reamplification process, especially when two rounds of reamplification are required to obtain a sufficient amount of DNA for cloning. When these polymerase chain reaction (PCR) fragments are cloned into plasmids, the inserts may have a significant proportion of unrelated sequences, leading to difficulty in isolating positive clones. Development of suitable methods to identify the specific cDNA clones from differential display of mRNA is highly desirable.

Recently, two methods have been described to overcome the problem of contaminating sequences. The first method uses Northern blotting to affinity-capture cDNA fragments. The labeled cDNA fragments hybridizing to