Rapid isolation of long cDNA clones from existing libraries

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Submitted February 5, 1991

Obtaining full-length or even near full-length cDNA clones has been a time-consuming and labor-intensive step in the analysis of cloned genes. Recent methods which use PCR as a preparative tool for cloning (1, 2) have made this step considerably more rapid, but may introduce sequence errors in the resulting clones due to numerous sequential rounds of in vitro replication. We describe a method for identifying long cDNA clones from existing cDNA libraries using PCR purely as a diagnostic tool.

The method is shown schematically in Figure 1. Sequence information from the 5′ end of an existing clone is used to design a gene-specific PCR primer. This is used in combination with a primer that abuts the 5′ cloning site in the vector to amplify a directionally cloned cDNA insert and then to reamplify the insert in the presence of ^{32}P and the absence of significant vector sequences to generate a radiolabeled 5′ end probe for screening libraries by plaque filter hybridization (3). For efficiency, we screen size-selected cDNA libraries at very high phage densities (200,000 to 1,000,000 pfu per 150 mm diameter plate). This density allows an entire library to be screened on relatively few plates. Crude phage plugs are picked into SM buffer. Each plug lysate contains a few hundred to a few thousand phage clones, one of which corresponds to the probe. Each lysate is then subjected to PCR between the gene-specific primer and a 5′ vector primer. The size of the amplification product shows the distance from the gene-specific primer to the 5′ end of the desired cDNA clone from each crude plug. Only cDNA clones of a desired length are purified and analyzed further. The principle limitations in this strategy are the quality of available libraries and the size range of reliable PCR amplification.

Results from two typical experiments are shown in Figure 2. 1C12 and 2F8 are clones from a large collection of cDNAs that represent RNAs expressed in the head but not in the early embryo of *D. melanogaster* (4). The initial clone of 1C12 is 1 kb in length

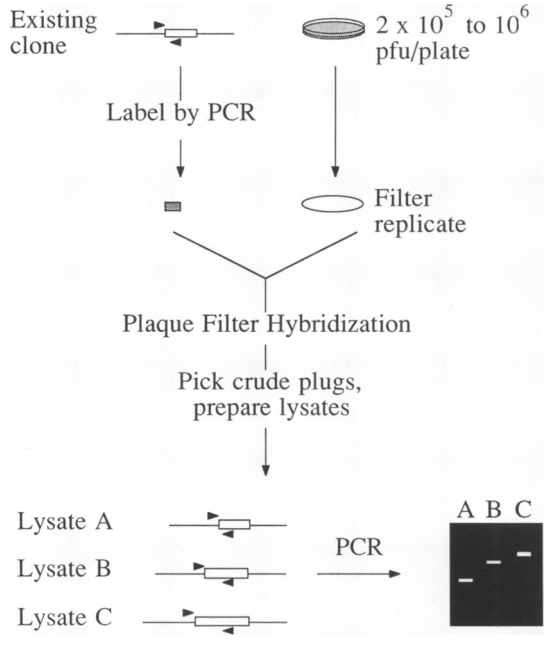

Figure 1. Flow diagram of screening procedure. PCR primers are represented as arrowheads. In each lysate only one phage clone, shown schematically, should contain sequences corresponding to the gene-specific primer. See text for full description. Detailed protocols available on request.

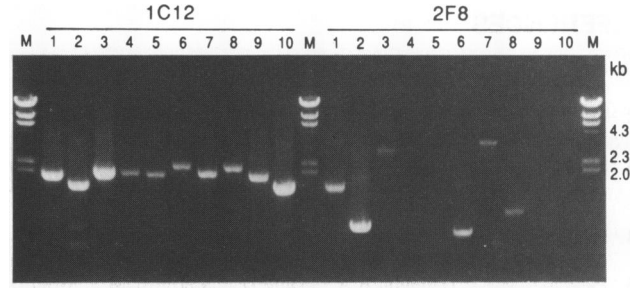

Figure 2. PCR products from two typical experiments. *Drosophila* adult head cDNA libraries in λEXLX (5), size-selected for cDNAs larger than 1.2 kb and 2.0 kb, respectively, were plated at 250,000 pfu/150 mm plate. Filter replicates were hybridized to ^{32}P-labeled cDNA inserts ($\sim 5\times10^9$ cpm/μug) from λSWAJ3 clones representing RNAs expressed in the adult head (4). Hybridization conditions were 50% formamide/5×SSPE/1×Denhardt's solution/1% SDS/100 μg ml^{-1} salmon sperm DNA at 42°C. Probe was added to $1-3\times10^6$ cpm/ml after 45 min. incubation in this buffer. Filters were washed 3 times in 0.1×SSPE/0.3% SDS at 50–55°C for 15–20 min. Plugs of phage corresponding to autoradiographic signals were picked with a baked Pasteur pipette or a broken 1 ml disposable serological pipette (outer diameter: 5 mm) into 200–300 μl SM buffer and allowed to resuspend for several hours. PCR was performed on 1 μl of each lysate in 40 μl reaction volume. Primers were used at 250 nM each. Amplifications were done with 40 cycles of 94°C, 45 sec.; 55°C, 45 sec.; 72°C, 3 min. PCR primers: SWAJ3.5, 5′-ATTTAGGTGACACTATAGAATACAC-3′; AG.5 (λEXLX), 5′-GCTGGTACCGGATCGAATTC-3′; 1C12, 5′- ATATTCCATGCCGTTTCGTG-3′; 2F8, 5′-CTTTGTCTCGTCATGCGTTG-3′.

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and hybridizes to a single poly(A)$^+$ RNA of approximately 5200 nt. Ten phage plug lysates from size-selected cDNA libraries were analyzed by PCR. The longest PCR product, from lysate 6, is 2.1 kb. The full length of the corresponding cDNA clone is 3.0 kb. The original 2F8 clone is 0.4 kb and hybridizes to several poly(A)$^+$ RNAs, ranging from 2500 to 6700 nt. The amplification product from lysate 7 is 3.4 kb and corresponds to a cDNA clone of 3.7 kb. The longest cDNA clone identified for each probe was subsequently plaque-purified, converted to plasmid in vivo (5), restriction mapped, and partially sequenced to verify its identity.

We have obtained comparable results with many other cDNA probes and with phage densities as high as 2×10^6 pfu per plate. Exposures of 24 hr or longer with an intensifying screen are usually necessary to detect hybridization signals on filters containing 10^6 or more pfu. While in our hands the efficiency of detection (hybridization signals/phage density) decreases with increasing phage density above 10^5 pfu per plate, the efficiency of screening (hybridization signals/filter) increases.

Abbreviations: kb, kilobase pairs; nt, nucleotide; PCR, polymerase chain reaction; pfu, plaque forming unit.

ACKNOWLEDGEMENTS

We thank M. Strathmann, K.Vijay Raghavan, and T.Wilke for constructive discussions and C.Mayeda and M.Whitney for excellent technical assistance. B.A.H. was supported in part by a USPHS Predoctoral National Research Service Award (T32 GM07616). This work was supported by a scholar's grant from the Lucille P.Markey Charitable Trust (M.J.P.) and USPHS Program Project Grant GM40499 (E.M.M.).

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