A simplified approach for isolating oligo(dT) primed cDNA clones with probes generated by cDNA selection

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The cDNA selection method has proven to be an effective approach to identify genes encoded in YACs (1–3) and has been used successfully in a number of positional cloning efforts (4–6). One problem with cDNA selection is that it produces short-fragment cDNA clones. Isolating individual short fragments, determining which are derived from different parts of the same gene, and isolating longer oligo(dT) primed cDNA clones corresponding to each gene are labor- and time-intensive steps.

To simplify the process of identifying long cDNA clones corresponding to the genes encoded in a YAC, we used the total mixture of selected short-fragment cDNAs as a probe to screen oligo(dT) primed cDNA libraries. First, we performed a reconstruction experiment. Oligo(dT) primed cDNA made from poly(A)⁺ RNA isolated from the Jurkat T cell line was cloned in the λ SH4K (7) vector. Approximately 400 plaque forming units from this cDNA library were plated in each of two plates, together with either 20 (Fig. 1A and B) or 40 (Fig. 1C and D) plaque forming units of a Charon BS (8) vector containing an oligo(dT) primed cDNA (CAT 53) that was encoded in YAC 225B1 (3). Duplicate filters were made for each plate. One of each pair of filters was hybridized to the CAT 53 probe (Fig. 1A and C). The other filter was hybridized either to a probe prepared with an uncloned mixture of cDNAs selected with DNA from YAC 225B1 (Fig. 1B), or to a probe generated with cDNAs amplified by PCR from the selected cDNA that had been cloned into λ gt10 (Fig. 1D). The same positive clones were detected with both the uncloned cDNA probe and the probe derived from the cloned cDNA (Fig. 1A and B; Fig. 1C and D). The CAT53 cDNA clone represented 3% of the short-fragment clones in the YAC 225B1 selected cDNA library. The selected cDNA mixture probe also detected two other cDNA clones which represented 10 and 20%, respectively, of the short-fragment clones in the selected cDNA library (data not shown).

We next performed a reconstruction experiment in which the number of cDNA plaques from the oligo(dT) primed cDNA library was increased. Plaque forming units (~3000) from the oligo(dT) primed Jurkat cDNA library were plated together with ~20 plaque forming units of an oligo(dT) primed cDNA clone (clone 27) isolated from a selected cDNA library from YAC 1220, which was derived from an X chromosome reference library

Figure 1. Comparison of filter hybridization with a single-copy probe and a selected cDNA mixture probe. Each filter contained ~400 plaques from a Jurkat cDNA library to which CAT53 clones were added (see text). Filters 1A and C were hybridized with the CAT 53 probe. Filter 1B was hybridized with the uncloned 225B1 selected cDNA mixture, and 1D hybridized with the 225B1 selected cDNA mixture that had been cloned into λ gt10.

database (ICRF, London). One of a pair of duplicate filters was hybridized to the clone 27 probe, and the other was hybridized to the selected cDNA mixture from YAC 1220. The selected cDNA was used directly after PCR amplification without having been cloned into λ gt10. Each plaque detected with the clone 27 probe was also detected with the mixed probe of selected cDNAs (Fig. 2A and B). In addition, the selected cDNA mixture probe detected two plaques marked by arrows (Fig. 2B) which were not detected by the clone 27 probe (Fig. 2A). The ends of each of the inserts in each of these two plaques were sequenced. The sequence did not correspond to any entry in GenBank. The two positive clones

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Figure 2. Screening of filters containing a large number of plaques. Each filter contained ~3000 plaques from the Jurkat cDNA library together with ~400 plaques of the cDNA clone 27. Filters 2A and B were a pair of duplicate filters. Filter 2A was hybridized with the clone 27 probe, and filter 2B hybridized with the YAC 1220 selected cDNA mixture probe that had not been cloned into λ gt10. The two new cDNA clones detected by the selected cDNA mixture probe were marked by arrows.

hybridized only to YAC 1220 DNA but not to any of four other YACs (Fig. 3 and unpublished results). These two clones are presumably unknown cDNAs encoded in YAC 1220 DNA.

Next, ~10 000 cDNA clones from the oligo(dT) primed Jurkat cDNA library were plated. One of the three replicate filters was hybridized to a cDNA clone (CAT53) derived from the YAC 225B1 selected cDNA library. The second was hybridized to the cDNA mixture probe selected from YAC 225B1, and the third was hybridized to a total human genomic DNA probe to detect human repetitive sequences. One positive clone detected with the clone CAT 53 was also detected with the selected cDNA mixture probe. The selected cDNA mixture probe also detected an additional four clones that were not investigated further but could be new cDNAs encoded by this YAC.

The results of these experiments demonstrate that a selected cDNA mixture probe can detect cDNA clones in an oligo(dT) primed cDNA library that are represented by $\leq 3\%$ of the clones in the selected cDNA mixture. The selected cDNA mixture probe detected several positive clones in filters, each of which contained as many as 10 000 plaques. In addition, the selected cDNA material that had not been cloned in λ gt10 proved to be as effective and specific as were the mixed cDNA inserts excised from the cloned library of selected cDNA fragments. This saves a laborious step in the application of cDNA selection to detect genes encoded in YACs.

The selected short fragments often contain a small amount of repetitive DNA so a counter-screen should be performed using Cot 1 DNA or an equivalent source of repetitive sequences as a probe against the cDNA library. With this precaution, the mixture of selected cDNA fragments has proven to be an excellent probe for screening cDNA libraries and identifying long clones corresponding to the relevant genes.

Figure 3. Hybridization of Southern blots containing YAC DNA to the new cDNA clones (see Fig. 2). Filter 3A and B were identical Southern blots. Each lane contained 1 μ g of DNA isolated from YACs after digestion with *Eco*RI. Lanes 1, 2 and 3 contained DNA from a control YAC, from YAC 1220 and yeast 1380 respectively. Filter 3A was hybridized with one of the new cDNA clones, and 3B with the other cDNA clone.

In a series of experiments, almost all cDNAs encoded by a series of YACs, including even those present at less than one part per million in the original libraries used for selection, were represented in the selected cDNA libraries. It may be possible to detect cDNA clones for almost all genes encoded in a YAC by performing cDNA selection with mixed cDNA libraries and using the selected material to screen normalized cDNA arrays. This procedure avoids several laborious steps such as the need to purify YAC DNA, to clone the selected cDNA fragments, to eliminate duplicate short fragment clones, or to identify which distinct short fragments are derived from different parts of the same mRNA.

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