A method for screening arrayed cosmid libraries with mega insert yeast artificial chromosomes

Martin H. Dreyling, Olufunmilayo I. Olopade and Stefan K. Bohlander*

Section of Hematology/Oncology, The University of Chicago, Chicago, IL 60637, USA

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Recent advances in the construction of yeast artificial chromosome (YAC) libraries have produced YAC libraries with an average insert size of about one million base pairs (Mbp). The CEPH Mega YAC library has also been partially ordered and mapped (1). Although these YACs allow the rapid cloning of large genomic regions, their size makes it difficult to analyze these regions in more detail (e.g. to find expressed sequences or to fine map interesting regions). Therefore, it is often necessary to convert a YAC into a cosmid contig to perform a more detailed analysis.

One strategy to convert a YAC into a cosmid contig is to screen a cosmid library directly with the YAC as a probe (2). However, this approach is fraught with several problems: (i) the hybridization has to be done under suppression conditions, i.e. in the presence of large amounts of unlabelled human DNA, because a YAC contains many repetitive elements; (ii) some method to separate the YAC DNA from the other yeast chromosomes has to be employed because the YAC constitutes only a small portion of the whole yeast DNA; (iii) the hybridization has to be very efficient, because each cosmid will constitute only a small portion of the probe (~4% in the case of a 1 Mbp YAC).

We have developed a method that allows the efficient screening of a gridded cosmid library using YACs of up to 1.6 Mbp in size as probes. The key to this method is the purification of the YACs with pulsed-field gel electrophoresis (PFGE) and the subsequent sequence-independent amplification (SIA) of the YAC DNA (3,4). This procedure provides an unlimited supply of PCR amplifiable DNA fragments derived from the YAC.

After pulsed-field gel electrophoresis the YAC bands were excised, and 4 vol TE was added to the agarose (SeaKem LE, FMC). The agarose was melted at 99°C for 3 min and 0.5 µl of the solution (containing 30–200 pg of YAC DNA) was used for SIA essentially as described (3). In the first four cycles of amplification T7 DNA polymerase (USB; Sequenase version 2.0) was used together with a primer that has a defined 5' sequence and a random pentanucleotide at the 3' end (5'-TGGTAGCTCTT-GATCANNNN-3'). These first cycles are performed at very low annealing temperatures (13–37°C) so that the 3' end of the primer can prime randomly. The subsequent cycles are performed using *Taq* DNA polymerase, high annealing temperatures and a second primer with the defined 5' sequence of the first primer (5'-AGAGTTGGTAGCTCTTGATC-3'). The resulting amplification products range in size from 300 to >1000 bp and represent the DNA from the excised YAC. 100 ng of the

spin-dialysed (Ultrafree spin filters; Millipore) amplified DNA were labelled with 100 µCi of [α -³²P]dCTP (3000 Ci/mmol; Amersham) using random oligonucleotide priming as described (5). Unincorporated nucleotides were removed by G50 Sephadex column chromatography. Repetitive sequences were blocked by preannealing the probe with 600 µg Cot1 DNA (BRL) and 600 µg sheared human placental DNA in 500 µl of hybridization solution [50% formamide, 1 M NaCl, 1% (w/v) sodium dodecyl sulfate (SDS), 10% (w/v) dextran sulfate, 50 mM NaPO₄ (pH 6.5)] at 45°C for 2 h.

A chromosome 9 flow-sorted cosmid library with 6-fold representation (Lawrence Livermore) had been spotted on 20 microtiter dish sized nylon filters with each microtiter well space containing a 4 × 4 array of cosmids. Each small 4 × 4 array contained one control plasmid. 1440 different cosmids were spotted on each of the 20 filters for a total of 28 800 cosmids in the whole library.

The cosmid filters were prehybridized for 2 h in hybridization solution (containing 80 µg/ml sheared denatured salmon sperm DNA). The preannealed probe was then added to 80 ml of hybridization solution and the filters were hybridized overnight at 42°C. The filters were washed in 0.1 × SSC (7.5 mM sodium chloride, 0.75 mM sodium citrate pH 7.5), 0.1% SDS for 1 h at room temperature, for 1 h at 65°C and exposed to X-ray film at -70°C overnight. Because filters were not stripped between hybridizations, the autoradiographs had to be compared with the films from previous hybridizations. Figure 1 shows the autoradiograph of one filter before and after the hybridization with a 800 kbp YAC probe (CEPH address: 886F9). Four new signals that resulted from the YAC hybridization can be easily identified.

We obtained 128 positive cosmids when we used the 0.8 Mbp YAC as a probe. Assuming an average cosmid insert size of 40 kbp and a 6-fold coverage of the cosmid library, one would have expected to detect 120 cosmids. The close agreement of the expected with the actual number of cosmid indicates that almost all the cosmids that correspond to this YAC were detected. One of the major concerns when using these SIA amplified YAC probes was that some portions of the YAC would not be well represented in the amplification products and that this would result in an incomplete detection of all the cosmids that correspond to the YAC. A more detailed analysis of the cosmids from a 200 kbp region within another YAC revealed that there were no gaps in the cosmid coverage.

* To whom correspondence should be addressed

Figure 1. (A) Autoradiograph of partial microtiter dish sized filter (columns 1-7; rows D-H) before hybridization with the YAC24 probe (0.8 Mbp). One can faintly see the 4×4 grid in each microtiter well space. (B) Autoradiograph of the same filter after hybridization with the YAC24 probe. Four additional signals can clearly be seen (D2, E3, G1, H7). Note that the upper left hand corner of each 4×4 grid contains a control plasmid.

We have successfully used YACs as large as 1.6 Mbp to screen the gridded cosmid library with this protocol. Several aliquots of these YAC probes can be easily generated by PCR amplification, which facilitates repeated hybridizations to confirm the original screening results. The described method should allow the rapid ordering of gridded cosmid and P1 reference libraries and should facilitate the integration of those libraries with the existing YAC libraries.

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