Rapid assembly of λ phage contigs within YAC clones

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The development of DNA cloning vectors capable of retaining large DNA inserts, such as yeast artificial chromosomes (YAC), has made a great contribution to the characterization of the human genome (1). YACs have been extensively used for large scale isolation of overlapping cloned regions of the human genome (2). However, their size and the difficulty of isolating the insert from the vector, and from the yeast background, represent drawbacks in the characterization of YAC clones.

One way to simplify the analysis of YACs is to subclone them into phage clones. We describe a method that allows the rapid assembly of λ phage clones derived from YACs into contigs of overlapping clones. This method is based on the serial hybridization of Alu-PCR probes obtained from λ phage plaques plated on numbered grids.

High molecular weight total yeast DNA was prepared from the YAC clones using the sucrose gradient method (1). We noticed that the DNA quality is of critical importance, since other methods, such as rapid preparation protocols, did not give comparable results. This might be due to the lower molecular weight of samples obtained with other methods due to shearing of DNA.

Partial digestion with Mbo I was performed by serial dilution of restriction enzyme as described in Sambrook *et al.* (3). After checking the digestion on an agarose gel, a sample with DNA fragment size ranging between 20 kb and 9 kb was chosen for the subcloning step. The partially digested DNA was filled-in with dGTP and dATP and ligated to predigested and partially filled in λ FIX II phage arms (Stratagene), as recommended by the manufacturer. A higher ligation efficiency was observed when dATP and dGTP were removed from the sample, using a Centricon-100 column (Amicon) followed by phenol extraction and ethanol precipitation. The ligated product was then packaged using the Gigapack II Plus kit (Stratagene). Phage clones were plated at low density (~1000 plaques per 150 mm plate) and filters were made following the plaque-hybridization procedure described in Sambrook *et al.* (3). Human clones were distinguished from clones containing yeast DNA by hybridization with total human DNA. At least one hundred (the number varying according to the size of the human insert in the YAC) human positive clones were picked from pure plaques and transferred to new plates in a gridded array. Duplicate filters were made from each grid.

Polymerase chain reaction (PCR) amplification was performed simply by touching the phage plaques with a toothpick which was then soaked in 50 μ l of TE, 2 μ l of which were then used

in the PCR reaction. Primers corresponding to Alu sequences were used in combination with vector primers as described in Nelson *et al.* (4). The Alu primers used were 557 and 558 (5) and vector primers were T3 and T7 promoter sequences. PCR was carried out using an automated thermal cycler (Perkin Elmer/Cetus) in a total volume of 50 μ l using the following conditions: 1 μ M each primer in 50 mM KCl, 10 mM Tris-HCl pH 8.0, 1.5 mM MgCl₂, 0.01% gelatin, 250 μ M each dNTP and 2 units of *Thermus Aquaticus* Polymerase (Perkin Elmer/Cetus); a denaturation step of 4 min at 94°C was followed by 35 cycles of 94°C denaturation (1 min), 50°C annealing (1 min), and 72°C extension (4 min), and by an extra extension step of 7 min at 72°C. PCR products were then analyzed by running them on an ethidium stained agarose gel. It is important to run an Alu-only and an Alu-vector reaction for each Alu and vector primer, in order to distinguish the end amplification products from the internal products derived from the amplification of two Alu sequences in opposite directions. Amplification products were obtained from approximately 95% of the clones, 60% of the clones gave at least one end-product and 30% gave products from both ends, using the above mentioned set of Alu primers.

PCR products were then run on a preparative low melting agarose gel and the bands corresponding to the amplification products were cut out and directly labelled with ³²P by the random hexamer method (6). Probes obtained in this way (preferentially Alu-vector PCR probes, but also Alu-Alu probes) were readily hybridized to the phage grid filters, to identify overlapping phage clones. Hybridization conditions were as follows: 1% SDS, 1 M NaCl, 10% dextran sulfate, 0.4 mg/ml sheared total human DNA. The filters were preassociated for 4-6 hours in the above solution at 65°C; the probe (106 cpm/ml final volume of hybridization solution) was then boiled for 10 min in 1 ml of the hybridization solution (containing human DNA), left to preanneal at 65°C for 30 min and eventually added to the hybridization bag. Two examples of the hybridization of the Alu-PCR probes to the grid are shown in Figure 1. Best results were obtained when probes longer than 500 bp were used.

After several serial hybridizations of Alu-PCR probes to the grid, a contig of λ phage clones from a YAC clone can readily be assembled. An example of such a contig from a YAC clone in the Xp22.3 region is shown in Figure 2. Approximately 35 hybridizations (26 of which were successful) were needed to assemble this contig. This YAC was previously found to span the breakpoint of two patients with an interstitial deletion in the Xp22.3 region (data not shown). The hybridization of Alu-PCR

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probes to the phage grid and subsequently to the patients' DNA allowed us to assign sequences corresponding to the patients' deletion breakpoints to λ clone 32 as shown in Figure 2.

The method described here proved to be very effective for the characterization of YAC clones. The contig shown in figure 2 was assembled in approximately one month. Elimination of time-consuming procedures such as plaque purification of λ phage clones, isolation of phage DNA, and subcloning of the insert termini, makes this method simple and fast. We have applied the method to several YAC clones mapping to regions of interest of the human genome.

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Figure 2. Contig of λ phage clones from a YAC clone in the Xp22.3 region. The asterisks represent either Alu-Alu or Alu-vector PCR probes; Alu-vector PCR probes can be distinguished from Alu-Alu probes since they are at the ends of λ clones. Probes 25 and 46 used in the hybridizations shown in Figure 1 are indicated by a ●. Dashed lines indicate overlaps as detected by hybridization. The size of the YAC and the phages are not to scale and have been arbitrarily drawn only in order to show the overlaps. A contig of approximately 50 λ clones has been assembled by Alu-PCR walking. The breakpoint of two patients has been identified within λ clone 32.

Figure 1. Hybridization of λ phage derived Alu-PCR probes to a numbered grid of 100 human λ clones grown in an ordered array. The probes correspond to Alu-Alu PCR products derived from λ clones 25 and 46, respectively. Several overlapping clones are identified with both probes. The faint positive signal of probe 46 on clone 12 was proved to be a hybridization artifact.