An easy and rapid method for the detection of chimeric yeast artificial chromosome clones

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Yeast artificial chromosome (YAC) clones are extremely valuable for studying large segments of genomic DNA. Experience with two libraries of human YAC clones (1, 2) indicates that the most common artifact is the presence of noncontiguous DNA segments in the same clone which may have arisen by co-ligation events or recombination between two YACs introduced into the same cell (3). It is estimated that the fraction of chimeric YAC clones in these libraries may approach 40–60% (3). In order to determine if a YAC clone is chimeric, one typically isolates the ends of the YAC insert and maps them to determine if they have discrepant chromosomal origin. However, the isolation of unique hybridization probes from the ends of YACs can be difficult. More recently attempts aimed at rapidly detecting chimeric YAC clones have involved the fluorescent *in situ* hybridization of *Alu*-PCR products from YAC DNA to human metaphase chromosomes. The limitation of this latter approach is that it requires expertise in preparing metaphase chromosomal spreads. An alternative method for detecting chimerism is described in this report. This method relies on the hybridization of the *Alu*-PCR product from YAC clones to a dot blot containing the *Alu*-PCR products from monochromosomal or highly reduced hybrids representing each of 24 different human chromosomes such as those previously described (4). For the specific amplification of human DNA from somatic cell hybrids, the PCR was carried out in a total volume of 100 μ l using 1 μ g of hybrid DNA and 0.3 μ M of *Alu* primer pDJ34 (5) in 10 mM Tris–HCl pH 8.3, 50 mM KCl, 1.25 mM MgCl₂, 0.01% (w/v) gelatin, 250 μ M each dNTP, and 2.5 units *Thermus aquaticus* polymerase (Perkin Elmer/Cetus). An initial denaturation step at 95°C for 5 min was performed and followed by 30 cycles of 94°C denaturation (1 min), 55°C annealing (1 min), and 72°C extension (4 min). Eighty microliters of each PCR reaction was precipitated and resuspended in 30 μ l of TE. One microliter was spotted onto a nylon membrane and the dot blots were prepared as previously described (4). The amplification of human DNA from YAC clones was carried out in a similar manner using primer pDJ34. The PCR product from the YAC insert was precipitated and 3 μ l was labelled with ³²P by the random primer method. The labelled probe was used at a concentration of 10⁶ cpm/ml of hybridization buffer and was preassociated with 0.25 mg/ml human placental DNA prior to adding to the filter. Hybridization washes were as described before (4). The filters were exposed to Kodak X-AR film for 45–90 min. Figure 1A illustrates the results of hybridization using a YAC that was known to have a contiguous segment of DNA based on mapping both ends of the insert to a somatic cell hybrid retaining chromosome 6p. The analysis using the hybridization scheme described above similarly

illustrated that this clone is nonchimeric as evident by hybridization to the hybrid retaining chromosome 6. The data in Figure 1B demonstrate a typical result obtained with a chimeric YAC; the YAC used in this experiment is chimeric with one segment from chromosome 1, and the other segment from chromosome 6. As is evident from this figure, one advantage of using this approach is that, in addition to detecting chimerism, it allows the identification of all the chromosomes to which the noncontiguous segments map which is very valuable for genome mapping activities. This approach has so far been applied to mapping over 25 YACs and has proven very sensitive for detecting chimeric clones. It is simple and rapid and can be extended to include regional mapping panels. For example, in the case of chromosome 6, we have prepared dot blots from various radiation hybrids to ensure that clones mapping to chromosome 6 do not contain sequences from different regions of this chromosome.

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Alu-PCR dot blot characterization of two YAC clones. (A) *Alu*-PCR dot blot hybridization of a non-chimeric YAC clone shows a positive signal to the chromosome 6 hybrid. Both ends of this YAC clone have been isolated by inverse-PCR and were confirmed to map to chromosome 6. (B) *Alu*-PCR dot blot hybridization of a chimeric YAC clone shows a positive signal to the chromosome 1 hybrid and to chromosome 6 defining the chromosomal origin of the two noncontiguous segments in this YAC clone. M = mouse, H = hamster.