SHORT COMMUNICATION

Fluorescence in Situ Hybridization of YAC Clones after Alu–PCR Amplification

CHRISTOPH LENGAUER,* ERIC D. GREEN,† AND THOMAS CREMER*

*Institut für Humangenetik u. Anthropologie, Universität Heidelberg, Im Neuenheimer Feld 328, 6900 Heidelberg, Federal Republic of Germany; and †Department of Genetics, Washington University School of Medicine, Saint Louis, Missouri 63110

Received November 5, 1991; revised February 10, 1992

Alu–PCR protocols were optimized for the generation of human DNA probes from yeast strains containing yeast artificial chromosomes (YACs) with human inserts between 100 and 800 kb in size. The resulting DNA probes were used in chromosome in situ suppression (CISS) hybridization experiments. Strong fluorescent signals on both chromatids indicated the localization of specific YAC clones, while two clearly distinguishable signals were observed in ≥90% of diploid nuclei. Signal intensities were generally comparable to those observed using chromosome-specific alphoid DNA probes. This approach will facilitate the rapid mapping of YAC clones and their use in chromosome analysis at all stages of the cell cycle. © 1992 Academic Press, Inc.

Yeast artificial chromosomes (YACs) (2) containing a variety of human inserts have recently been mapped by fluorescence in situ hybridization (FISH) using either total yeast clone DNA or YAC DNA isolated from pulsed-field gels (for review see (9, 13)). However, the hybridization efficiency of YACs was generally less than satisfactory in metaphase spreads, while interphase nuclei could not be evaluated at all. To overcome these problems, we have tested whether the Alu–PCR approach first described by Nelson et al. (10) would allow the amplification of human DNA sequences from YACs with complexities sufficient for FISH experiments. Alu primer 517 (nt positions 220 to 236 of the consensus Alu repeat) (10), which was successfully used to amplify human sequences from rodent × human hybrid cell lines (5, 8), did not yield DNA probes useful for in situ hybridization when applied to YAC clones (data not shown). It appeared necessary to search for an Alu primer pair and to define PCR conditions that would allow the specific amplification of human sequences between Alu blocks in all possible orientations with optimized yields from YAC clones.

For this purpose we designed two primers, CL1 and CL2, chosen from the most conserved regions of the Alu repeat family, which are located close to the 5′ and 3′ ends of the approx 300-bp-long Alu block, respectively (4): CL1, 5′ TCC CAA AGT GCT GGG ATT ACA G 3′ (nt positions 23 to 44 of the consensus Alu repeat); CL2, 5′ CTG CAC TCC AGC CTG GG 3′ (nt positions 244 to 260). Primer 278 described by Nelson et al. (10) is partially identical with primer CL1 but contains 11 additional bases at the 5′ end. Due to the orientation of these primers, Alu–PCR-amplified sequences would contain only very brief Alu segments. This may facilitate the effective suppression of labeled Alu sequences in FISH experiments. For comparison, several other primers were derived from regions located more interior within the Alu block, including primers CL3, 5′ GTT GGC CAG GCT GGT CT 3′ (nt positions 82 to 98); and TC 65 (nt positions 220 to 236) (10), as well as primer A1 (nt positions 268 to 285) (1), located at the extreme 3′ end of the Alu repeat.

Under a variety of amplification conditions, the primer pairs CL1/CL2, CL1/TC65, CL1/A1, CL3/CL2, CL3/TC65, and CL3/A1 were tested for their ability to amplify a maximum amount of human sequences from YAC clones; amplification products from yeast DNA should be avoided. For PCR experiments, 100 ng of purified genomic yeast clone DNA was used. Alternatively, agarose plugs prepared for pulsed-field gel electrophoresis containing the respective yeast cells and stored in 0.5 M EDTA were equilibrated two times, 30 min each, in 1 M Tris–HCl (pH 8.0) and 30 min in PCR buffer (10 mM Tris–HCl, pH 8.4, 50 mM KCl, 1.5 mM MgCl2, 0.001% gelatin) at room temperature. Afterwards the plugs were stored overnight in PCR buffer at 4°C. The plug was melted for 5 min at 70°C, and an aliquot equivalent to approx 100 ng of genomic yeast cell DNA was added to the Alu–PCR assay. For rapid testing we were also able to pick a small amount of yeast cells (just visible on a sterile pipet tip) directly from clones grown on plates. After dilution of the cells in 1.5 ml distilled water, aliquots of up to 20 µl were added to the PCR assay without any further pretreatment. This latter approach, however, provided fewer reproducible amplification yields.

In the PCR assay, each primer was used at a concentration of 0.25 µM in a total volume of 100 µl PCR buffer containing 250 µM of each of the four dNTPs and 2.5 units of Taq polymerase (Perkin–Elmer/Cetus). After initial denaturation at 96°C for 3 min, 30 cycles of PCR were carried out with denaturation at 96°C for 1 min and extension at 72°C for 6 min. The annealing time was 30 s, while the annealing temperature was varied between 55 and 37°C. Ten-microliter aliquots of amplified DNA sequences were fractionated by electrophoresis in a 1.5%
FIG. 1. Agarose gel (1.2%) of Alu–PCR-amplified YAC clones of the CF contig (3). Lanes 1, 2: two independent Alu–PCR amplifications of a melted agarose plug containing the YAC yCF-1/7/5-R (790 kb). Lanes 3–9: Alu–PCR-amplified YAC clone DNA, yKM19-3 (150 kb); yCF-10 (240 kb); yCF-7 (240 kb); yCF-5 (280 kb); yW30-5 (260 kb); yJ311-3 (340 kb); yJ311-1 (350 kb); marker lane A, λ/HindIII; marker lane B; 123-bp ladder. Overlapping YAC clones show bands with apparently identical position and intensity, while other bands are distinctly different.

agarose gel in 1× TAE (0.04 M Tris-acetate, 0.001 M EDTA). The highest yield of amplification products was obtained at the lowest annealing temperature (37°C) (data not shown). In particular, at decreasing annealing temperature a smear of amplification products ranging up to 8 kb became more prominent. Primer combinations CL1/CL2 and CL3/CL2 showed the best yield of amplification products. Figure 1 shows a typical agarose gel with Alu–PCR-amplified sequences obtained with CL1/CL2 from eight YAC clones containing human inserts between 150 and 790 kb, which belong to a YAC contig previously established from the cystic fibrosis gene at band 7q31 (3). The size and intensity of the individual bands were highly reproducible for each YAC clone. None of the combinations of primer pairs chosen in these experiments yielded specific amplification products from genomic yeast DNA, although combinations containing primer CL3 showed a band <50 bp indicating the formation of primer dimers. Accordingly, we decided to test amplification products obtained with primer pair CL1/CL2 for their suitability as FISH probes.

PCR products were ethanol precipitated, resuspended in double-distilled water, and used for nick translation with biotin–11-dUTP. Alternatively, biotin–11-dUTP was directly incorporated during the PCR. Note that the size of the labeled probes should range between some 100 and 400 bp for efficient FISH with low background. In the case of PCR labeling, the amplification products should be post-treated with appropriate concentrations of DNase I. Chromosome preparations prepared from phytohemagglutinin (PHA)-stimulated human blood lymphocytes using standard techniques were pretreated with RNase A and pepsin, postfixed in 1% acid-free, buffered formaldehyde, and stored in 70% ethanol overnight according to the protocol described by Ried et al. (12). Chromosomal in situ suppression (CISS) hybridization and probe detection with fluorescein isothiocyanate (FITC) conjugated to avidin were carried out as described previously (7) with the following modifications: For hybridization 100–150 ng of the Alu–PCR-amplified YAC DNA was used as a probe after preannealing with titrated amounts (30–100 μg) of unlabeled Cot 1 DNA (BRL/Life Technologies, Cat. No. 5279SA). The signals

FIG. 2. Metaphase spread (A) and interphase nuclei (A, B) after CISS hybridization of the Alu–PCR-amplified YAC clone yCF-1/7/5-R and detection of the biotinylated probe with avidin FITC. Chromosomes counterstained with propidium iodide.
were amplified once (11). Cells were counterstained with 1 µg/ml 4,6-diamidino-2-phenylindole-dihydrochloride (DAPI) and 0.2 µg/ml propidium iodide, mounted in fluorescence antifading buffer (1 mg p-phenylenediamine in 1 ml glycerine buffer, pH 8.0), and evaluated with a Zeiss Photomicroscope III.

Figure 2A shows the result of CISS hybridization of Alu-PCR-amplified sequences from YAC clone yCF-1/7/5-R to a normal male human metaphase spread. Chromosome identification was performed after DAPI banding or after simultaneous hybridization with a chromosome 7 alploid repeat probe (data not shown). In 100 subsequent metaphase spreads, which were analyzed on two slides, strong signals with the expected localization on the long arm of chromosome 7 were seen on both chromatids. Figure 2B demonstrates that the Alu-PCR probe obtained from this YAC clone also yielded strong specific signals in interphase nuclei. Table 1 shows the results of signals counted in interphase nuclei of normal lymphocytes after CISS hybridization with Alu-PCR-amplified probes obtained from five YAC clones. For each clone, 90% or more of randomly evaluated nuclei showed two clearly separated signals.

Possible limitations of this approach should be taken into consideration. The efficiency of probe generation by Alu-PCR depends on the number of adequately spaced Alu elements that flank site-specific sequences in each individual YAC. Therefore, YAC clones derived from parts of the genome with a particularly low content in Alu sequences may not be used effectively. Up to now we have tested more than 60 YAC clones containing some 100 to 800 kb of human insert from various regions of the human genome, including both R- and G-bands. Each of these clones yielded clearly detectable metaphase and interphase signals using the protocol described in this report. While many clones showed signals restricted to one specific chromosome band, others showed signals on different chromosome subregions indicating that these clones either detected sequence homologies within the respective subregions or contained a chimeric YAC or several different YACs. Alu-PCR-generated probes from chromosome band-specific YAC clones provide ideal tools for the analysis of specific numerical and structural chromosome aberrations at any stage of the cell cycle (6).

ACKNOWLEDGMENTS

We thank Angelika Wiegensstein for photographic work. This work was supported by grants from the Deutsche Forschungsgemeinschaft (Cr 59/10-1). C.L. is the recipient of a scholarship from the Konrad-Adenauer-Stiftung. E.D.G. is a Lucille P. Markey Scholar, and his work was supported in part by a grant from the Lucille P. Markey Charitable Trust.

REFERENCES


<table>
<thead>
<tr>
<th>YAC clone</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>yCF-1/7/5-R</td>
<td>1</td>
<td>4</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>yCF-10</td>
<td>3</td>
<td>5</td>
<td>90</td>
<td>2</td>
</tr>
<tr>
<td>yCF-7</td>
<td>1</td>
<td>1</td>
<td>92</td>
<td>4</td>
</tr>
<tr>
<td>yCF-5</td>
<td>2</td>
<td>4</td>
<td>91</td>
<td>3</td>
</tr>
<tr>
<td>yW30-5</td>
<td>0</td>
<td>1</td>
<td>97</td>
<td>2</td>
</tr>
</tbody>
</table>

Note. Number of evaluated nuclei: 200.