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# Fluorescence *in situ* hybridization with *Alu* and L1 polymerase chain reaction probes for rapid characterization of human chromosomes in hybrid cell lines

**(interspersed repetitive sequence elements/chromosome banding/composite hybridization probes/chromosomal *in situ* suppression hybridization)**

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**ABSTRACT** **Human–rodent hybrid cell lines have been analyzed with regard to their human DNA content by using various DNA probe sets, derived from the hybrids, for *in situ* hybridization to normal human metaphase chromosome spreads. Total genomic hybrid DNA was compared with probe sets of hybrid DNA that were highly enriched in human sequences. The latter probes were obtained by amplification through the polymerase chain reaction (PCR) using oligonucleotide primers directed to human specific subsequences of the interspersed repetitive sequences *Alu* and L1. Previously unidentified chromosomal material within hybrid lines was characterized with speed and precision. It is demonstrated that the complete human complement of hybrid lines can be rapidly assessed by comparing the data obtained with the *Alu*-PCR products with the results from the L1-PCR products or from the genomic hybrid DNA. This approach using interspersed repetitive sequence-PCR products is simple and fast and also provides an alternative way of generating complex DNA probe sets for the specific delineation of entire chromosomes or subchromosomal regions by *in situ* hybridization.**

Human–rodent somatic cell hybrid lines are very important tools for the analysis of the human genome. In all applications it is crucial to know the complete human chromosomal complement of these lines. The lines are often unstable with regard to chromosomal content and arrangement and, therefore, require periodical re-examinations. Biochemical analysis or Southern blot analysis using large sets of DNA probes is labor-intensive and, in general, an exact determination of the human DNA content is not feasible with these methods. To obtain comprehensive data, cytogenetic analysis is the method of choice. G11 staining of metaphase chromosomes is used to differentiate human from rodent chromosomes on the basis of color (1), but G11 staining is of limited use for the accurate identification of the human chromosomal material. Assignment can be achieved by chromosome banding techniques, which are, however, labor-intensive and particularly difficult when structural changes, including deletions and translocations of small chromosomal regions, are present.

*In situ* hybridization with total human genomic DNA as the probe against metaphase chromosomes of human–rodent hybrid cells results in a highly specific staining of the entire human chromosomal complement (2–5); however, no chromosomal assignment of the labeled chromatin is achieved. By using *Alu*-DNA as the probe, a pattern of hybridization signals similar to negative Giemsa banding is produced (6–8). Complete human chromosomes are clearly visible above the rodent background, and these can be identified by the *Alu*-banding pattern (P.L. and D.C.W., unpublished data). However, small rearranged chromosomal material is still very difficult to analyze.

Reverse *in situ* hybridization is an alternative approach to overcome those limitations (9, 10). When total hybrid cell DNA is used as a probe against normal human metaphase chromosome spreads, under conditions that suppress signal from ubiquitous repetitive DNA (11–15), the human DNA present in the hybrid line is specifically delineated. However, because the percentage of cells with human chromosomal material within hybrid lines varies, optimization of the hybridization conditions is required for each cell line or subculture, respectively. Furthermore, certain DNA sequences with shared homology to DNA of other chromosomes, such as pericentric repetitive DNA, may cause considerable cross-hybridization to chromatin regions that are not present in the hybrid line.

Recently, methods have been developed to specifically amplify human DNA within interspecies hybrid-cell DNA (16, 17). DNA oligonucleotides that target human-specific subsequences of the *Alu* and L1 repetitive DNA elements, respectively, are used as primers in polymerase chain reactions (PCRs). The human DNA between two repetitive DNA elements is amplified when these elements are located appropriately with regard to distance and orientation. Here we compare the utility of genomic DNAs and interspersed repetitive sequence PCR (IRS-PCR) products from hybrid cell lines to analyze their human DNA content by *in situ* hybridization.

## MATERIALS AND METHODS

**Cell Lines.** Four human–hamster somatic cell hybrid lines containing a single intact human chromosome as shown by cytogenetic analysis were used in this study. Hybrid lines E1 and K1 were derived by 6-thioguanine selection of hybrids containing the human X chromosome plus other human chromosomes on a RJK88 Chinese hamster background. Analysis of 10 Giemsa-banded and 20 G11-stained metaphase cells showed the E1 cell line to contain chromosome 7 and the K1 cell line to contain chromosome 22 as the only human chromosomes. Both hybrids have been submitted to the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Camden, NJ; E1 = GM10790, K1 = GM10888).

Hybrid TS-1 has been described (18) and contains an intact human chromosome 18 and an unidentified metacentric marker chromosome of human origin in all cells examined.

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Abbreviations: IRS, interspersed repetitive sequences; PCR, polymerase chain reaction; CISS, chromosomal *in situ* suppression; DAPI, 4′,6-diamidino-2-phenylindole.

Hybrid EyeF3A6 (GM10027) has been described (19) as containing chromosome 22 as its only intact human chromosome. Conventional cytogenetic analysis by us and at the Human Genetic Mutant Cell Repository showed chromosome 22 in all cells, and one or two unidentified pieces of human chromosome material translocated to hamster chromosomes in each cell.

**Chromosome Preparation.** Metaphase chromosome spreads from stimulated human lymphocytes were prepared by standard techniques of colcemid treatment, hypotonic shock, and methanol/acetic acid fixation, as described (13). For *in situ* hybridization reactions, chromosomes were denatured in 70% (vol/vol) formamide/2× SSC (1× SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7) for 2 min at 68°C, followed by incubation in 70%, 90%, and 100% ice-cold ethanol (3 min each) and air drying.

**DNA Probes.** Genomic DNA of the hybrid cell lines was isolated as described (20). For PCR, 1 μg of hybrid DNA was used in 100 μl of 1 μM *Alu* primer 517 (17) or L1Hs primer (18)/10 mM Tris·HCl/50 mM KCl/1.2 mM $MgCl_2$/0.01% gelatin/250 μM each of the four dNTPs and 2.5 units of *Thermus aquaticus* (*Taq*) DNA polymerase (Perkin–Elmer/Cetus). After initial denaturation at 95°C for 5 min, 30 cycles of PCR were carried out with denaturation at 94°C for 1 min, hybridization at 55°C for 1 min, and extension at 72°C for 4 min (last extension, 7 min). The products of each reaction were incubated with 1 unit of the large fragment of DNA polymerase I (Klenow fragment) at 37°C for 20 min. After enzyme inactivation at 65°C for 10 min, the DNA was ethanol precipitated. The pellet was resuspended in 60–70 μl of 10 mM Tris·HCl/1 mM EDTA. PCR products were analyzed by agarose gel electrophoresis.

Previously mapped DNA clones were used in cohybridization experiments to confirm chromosome assignment: cosmid clone 519 (21) located on 21q (provided by P. Watkins, BRL/Life Technologies); cosmid clone C31 (gift from I. Encio and S. Detera-Wadleigh, National Institutes of Health) containing the human glucocorticoid receptor gene, which is located on 5q (22); phage clone 7-1-558 containing sequences of the human immunoglobulin variable region of the heavy-chain locus (23) located on 14q (provided by F. W. Alt, Columbia University); phage clone FIX (gift from R. M. Kotin, Cornell University Medical College, and M. Siniscalco, Sloan–Kettering Institute) containing adeno-associated virus sequences that recognize specifically the cellular integration site on human 19q (24).

All probe DNAs, genomic hybrid cell DNA, *Alu*-PCR or L1-PCR products, and all but one previously mapped DNA probes were labeled with biotin by nick translation (25). Cosmid 519 was labeled with digoxigenin-11-dUTP (Boehringer Mannheim) by nick translation (26).

***In Situ* Hybridization and Probe Detection.** Chromosomal *in situ* suppression (CISS) hybridization conditions were done as described elsewhere (13). Briefly, 2 μg of genomic hybrid DNA was combined with 2 μg of total Chinese hamster DNA, 2 μg of total human DNA, and 4 μg of salmon sperm DNA, ethanol precipitated, and resuspended in 10 μl of hybridization mixture [50% formamide/1× SSC/10% (wt/vol) dextran sulfate]. Alternatively, when *Alu*-PCR or L1-PCR products were used as probe, 100 ng or 400 ng of probe, respectively, was combined with various combinations of competitor DNA: 2 μg of human and 8 μg of salmon sperm DNA, 10 μg of human DNA, 10 μg of human $C_0t$ 1 DNA (BRL/Life Technologies; catalog no. 5279SA) or 10 μg of salmon sperm DNA. Probe denaturation, prehybridizing, and hybridization were done as described (13). After posthybridization washes and blocking with bovine serum albumin, probes were detected by means of fluorescein isothiocyanate (FITC) conjugated to avidin. Probe 519 was detected by indirect immunofluorescence by means of Texas red-conjugated antibodies (26). No signal amplification procedure was applied. Chromosomes were counterstained with propidium iodide. Microscopic slides were evaluated by conventional epifluorescence microscopy. Digital images of specimens were generated as described (26).

## RESULTS

To compare the efficiency of total genomic DNA and IRS-PCR products for analyzing the human DNA complement of human–hamster hybrid cell lines, four independent hybrid lines were tested in a blind fashion. Probe sets were labeled with biotin and hybridized to normal human metaphase chromosomes followed by standard detection procedures using fluorescein-labeled avidin. The IRS PCR primers used in this study are expected to amplify DNA of different complexity. The *Alu* primer 517 was chosen because it usually results in a higher number of amplification products than other *Alu* primers. However, because of its location within the *Alu* repeat (16), this primer yields PCR products that contain high levels of *Alu* sequences. Therefore, we tested *Alu*-PCR products under CISS hybridization conditions by using different competitor DNAs (total human DNA at 0.2 mg/ml or 1 mg/ml, or human $C_0t$ 1 DNA at 1 mg/ml). A specific signal could only be achieved with $C_0t$ 1 DNA as competitor, whereas total human DNA was not sufficient to suppress the *Alu* sequences within this probe set. The location of the L1 primer at the 3′ end of L1 repeats suggests that the L1-PCR products contain predominantly single-copy sequences. However, CISS hybridization with and without human competitor DNA showed that there are repetitive sequences (most likely *Alu* sequences) amplified, and therefore human competitor DNA is required to achieve specific labeling.

The *Alu*-PCR products of two hybrid lines, designated GM10790 and GM10888, each labeled specifically only one chromosome, number 7 or 22, respectively (Fig. 1 *A* and *C*). Chromosome assignment was achieved by 4′,6-diamidino-2-phenylindole (DAPI) banding for GM10790. In the case of GM10888, in addition to DAPI staining, cohybridization of the probe set with cosmid clone 519, previously mapped to chromosome 21 (data not shown), was carried out to distinguish between chromosomes 21 and 22. The target chromosomes are not evenly decorated but show an R-banding-like pattern correlating with the occurrence of *Alu* sequences along the chromosomes (Fig. 1 *B* and *D*). The labeling of chromosome 7 in hybrid line GM10790 corresponds to the result seen with GM10790 genomic DNA. However, although the GM10888 genomic DNA probe revealed extensive labeling of chromosome 22, additional signals were seen in the centromeric region and on the short arms of the other D- and G-group chromosomes (chromosomes 13, 14, 15, and 21; compare cell line GM10027 below). Similar results were seen with the L1-PCR products. These additional signals are probably due to known cross homologies of sequences in these chromosomal regions (e.g., refs. 27–31) and therefore do not reflect parts of the genome present in the hybrid line. The *Alu*-PCR products of line GM10888 did not label the centromeric region or the short arm of chromosome 22 (Fig. 1*D*), most likely because of an underrepresentation of *Alu* sequences in this region (8); hence, there is no cross-hybridization to the other acrocentric chromosomes. The L1-PCR products of GM10888 label the entire chromosome 22, again generating a banding pattern, including the short arm of 22 (Fig. 1*E*).

The analyses of hybrid cell lines TS-1 and GM10027 showed a more complex pattern with regard to the human DNA content. With total genomic DNA of hybrid line TS-1, strong fluorescent hybridization signals were observed decorating human chromosome 18 and the short arm of chro-

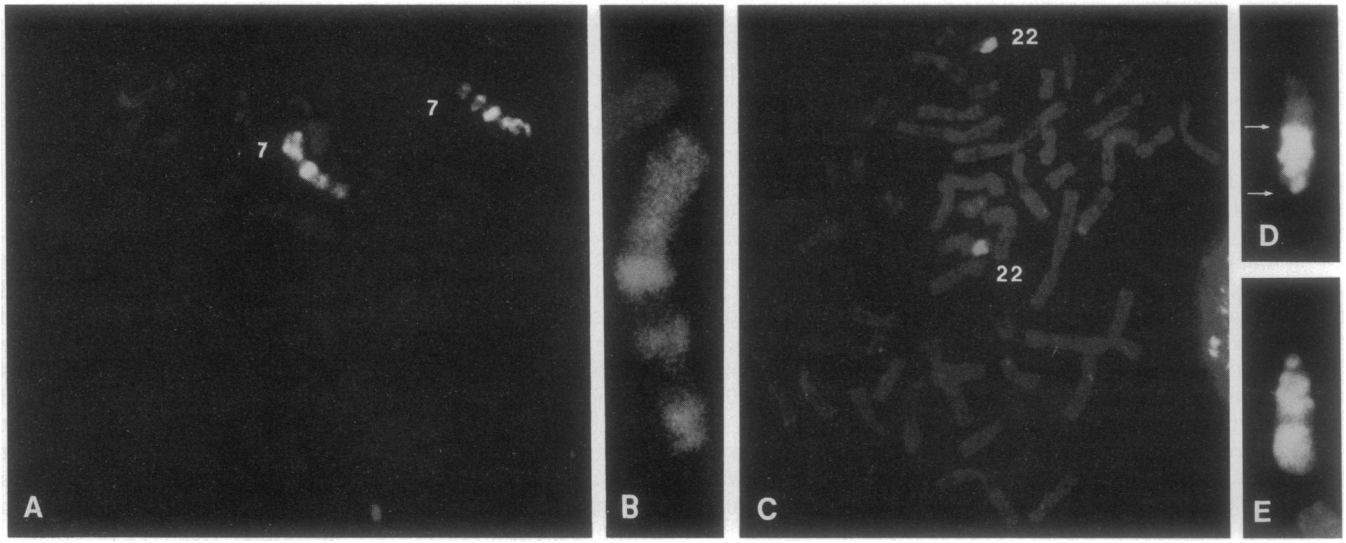


FIG. 1. CISS hybridization with IRS-PCR products, derived from hybrids GM10790 and GM10888, to normal human metaphase chromosomes. (*A*) *Alu*-PCR products of GM10790 specifically label the two homologous chromosomes 7 within a metaphase chromosome spread; note the banding pattern of the label. (*B*) Same experiment as in *A* showing one labeled chromosome 7 with the R-banding-like signal pattern (see text for details). (*C*) Specific labeling of human chromosome 22 in a metaphase chromosome spread with the *Alu*-PCR products from hybrid line GM10888 as the probe set. (*D*) Same experiment as in *C* showing the nonuniform labeling of chromosome 22. The *Alu*-PCR probe does not label the centromeric region and the short arm of chromosome 22. The labeled region of the long arm is depicted by arrows. (*E*) Chromosome 22 after labeling with the L1-PCR probe set from GM10888. Note the banded signal spanning the entire chromosome (see text for details).

mosome 5 (Fig. 2*A*). To confirm the chromosome assignment for the second signal on the short arm of chromosome 5, cohybridization was carried out with DNA clone C31, previously mapped to the long arm of chromosome 5 (Fig. 2*B*). A retrospective analysis of Giemsa-banded hybrid cells indicated that the previously unidentified marker chromosome of TS-1 is an iso(5p) chromosome. Additional hybridization signals, again most likely due to cross homology, were seen at the centromeres of several other chromosomes as well.

Both the *Alu*-PCR or L1-PCR products of TS-1 resulted in a highly specific delineation of chromosome 18 and 5p (Fig. 2 *B* and *E*). The *Alu*-PCR products did not stain the centromeres of chromosomes 5, 18, or any other chromosome, whereas the L1-PCR products stained the centromeric regions of chromosomes 5 and 18, but no additional centromere labeling was found. Thus, the IRS-PCR products resulted in highly specific hybridization signals without any significant cross-hybridization.

When using genomic DNA of hybrid cell line GM10027 as probe (see Fig. 2*D*), complete decoration of chromosome 22 and substantial labeling of chromosomes 19 (which is more strongly labeled on the short arm than on the long arm), 15 (short arm and proximal third of the long arm), and 14 (short arm) was observed. In addition to the predominantly labeled centromeres of these four chromosomes, again several other centromeres were labeled. Cohybridization experiments with probes previously mapped to 14q and 19q, respectively (see *Materials and Methods*), were used to confirm the identity of chromosomes exhibiting hybridization signals (data not shown). To identify chromosome 15 DAPI staining was applied. Fig. 2*E* shows the result using the *Alu*-PCR product of GM10027 with the labeling of the long arm of chromosome 22, the short arm of 19, and the proximal third of the long arm of 15. Thus, not only the centromeres but also the regions of 22p, 15p, and 14p are spared from decoration with the *Alu*-PCR probe. The staining of the short arm of chromosomes 14 and 15 with the genomic DNA probe (Fig. 2*D*) can be interpreted as cross homologies of sequences on 22p. These data demonstrate that the previously unidentified translocated chromosome pieces in GM10027 can be attributed to the proximal part of 15q and to 19p. It should be noted that the *Alu*-PCR products from both cell lines containing chromosome 22 do not hybridize to 22p (compare Fig. 1 *C* and *D* with Fig. 2*E*).

In conclusion, to obtain comprehensive data with regard to the human DNA content in human–rodent hybrid cell lines, comparison of the *in situ* hybridization results obtained with two probe sets, *Alu*-PCR products and genomic hybrid DNA or *Alu*-PCR and L1-PCR products, respectively, should prove to be a very powerful method for such karyotyping analysis.

## DISCUSSION

The use of IRS-PCR products of human–rodent cell hybrid lines for reverse *in situ* hybridization experiments allows a rapid and direct assessment of the human DNA complement in the hybrid lines. By using appropriate competitor DNA the genomic regions present in the lines are labeled in a highly specific manner, even when chromosomal subregions are to be identified. This approach is very fast and more efficient than the use of genomic hybrid-cell DNA as a probe because the DNA probe set is highly enriched with human sequences. Furthermore, the additional hybridization signals often seen with genomic hybrid DNA—mainly in centromeric/pericentric regions and on the short arm of the human acrocentric chromosomes—can be avoided. A comparison of the results obtained with *Alu*-PCR probes and L1-PCR probes assists in determining which additional DNA sequences are truly represented in the hybrid line.

Neither *Alu*- nor L1-PCR products evenly decorate the target chromosomes, but they generate a quasi-banded pattern, in part, because of the selective amplification of certain DNA fragments by the IRS-PCR procedure. A second major contributing factor is the uneven distribution of the corresponding repetitive DNA elements along the chromosomes. L1 repeats occur dominantly in Giemsa dark bands, whereas *Alu* elements are predominantly located in Giemsa light bands (6–8). Therefore the two IRS-PCR products of one hybrid cell line, used separately and/or in combination, will give a comprehensive picture of the human chromatin material present in the hybrid line. Using PCR products primed

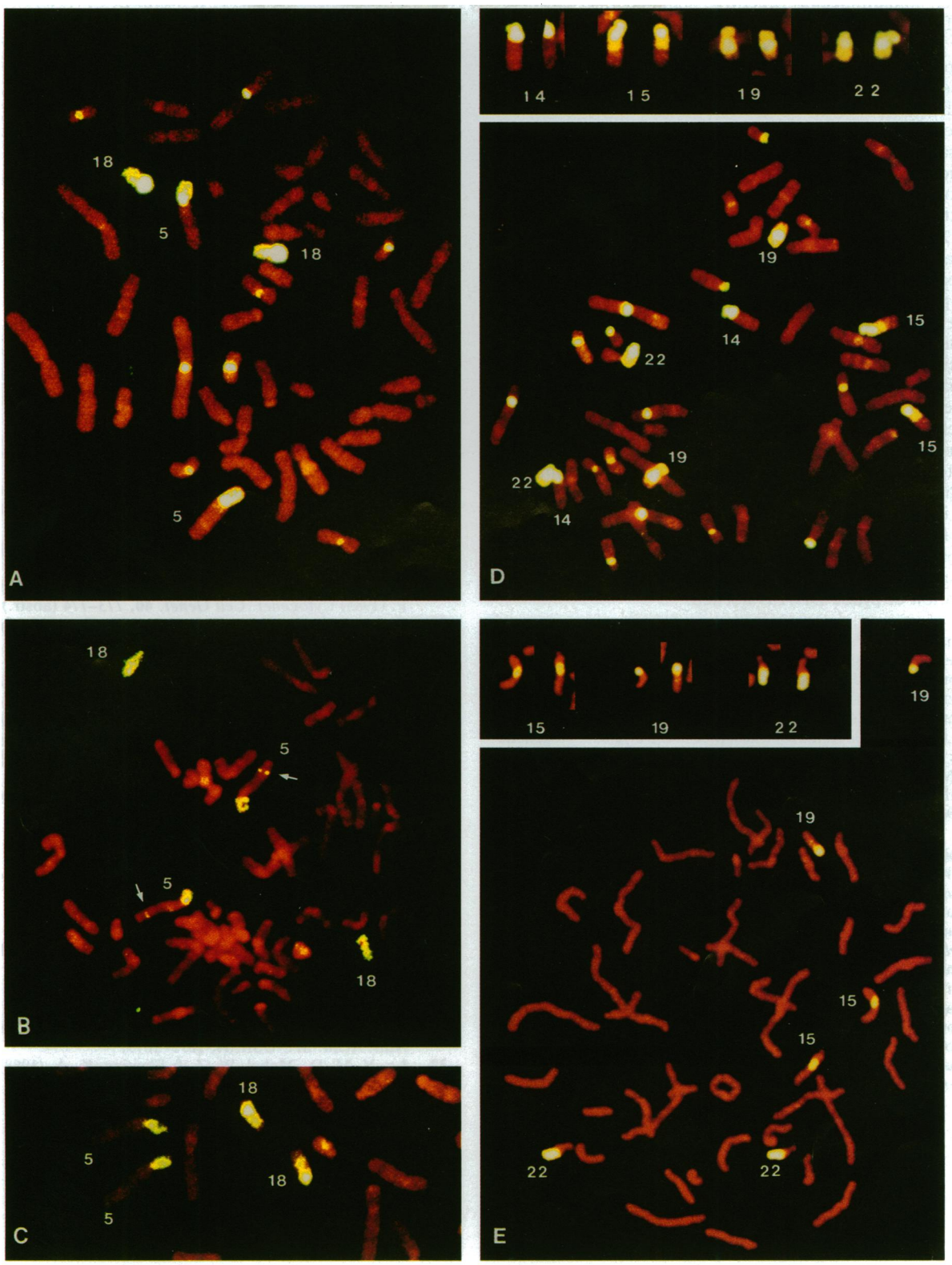


FIG. 2. CISS hybridization with various probe sets from hybrid lines TS-1 (*A–C*) and GM10027 (*D* and *E*) to normal human metaphase chromosomes. (*A*) Total hybrid DNA of TS-1 as the probe decorates chromosome 18 and 5p as well as four additional centromeric regions. (*B*) Specific delineation of 18 and 5p by the *Alu*-PCR probe set derived from TS-1. No additional centromere labeling is found. In this experiment cohybridization with clone C31 (signals depicted by arrows) was done to confirm the identity of chromosome 5. (*C*) L1-PCR products of TS-1 label the same chromosomal regions as *Alu*-PCR products but show different banding pattern of the signal. (*D*) Genomic DNA of GM10027 as the probe results in a complex picture of labeled chromosomal material. Besides numerous signals in centromeric regions and on short arms of acrocentric chromosomes, chromosome 22 is entirely labeled, and chromosomes 14, 15, and 19 are significantly labeled. (*Inset*) Chromosomes 14, 15, 19, and 22 of the same metaphase. (*E*) *Alu*-PCR probe set demonstrates the presence of 19p and the proximal portion of 15q in addition to chromosome 22 in GM10027. (*Inset*) Chromosomes 15, 19, and 22 of the same metaphase.

by other human specific repetitive sequences or combinations of primers from different IRSs in a PCR reaction could further improve the analysis. The G- and R-banding-like signal pattern with L1- and *Alu*-PCR products, respectively, can also be instrumental for identifying the labeled chromosomal DNA (compare Fig. 1*B*).

Gel electrophoresis of the IRS-PCR products from cytogenetically characterized human monochromosomal hybrid lines indicates that the DNA fragment profile of each human chromosome differs distinctly (16, 17). By using IRS-PCR products as probes in Southern blot experiments with hybrid-panel DNA, chromosomal assignment can be achieved. Incorporation of nonisotopically labeled nucleotides during the PCR reaction (32) could both facilitate the *in situ* hybridization experiments and provide an alternative method of confirming the *in situ* results by Southern blot analysis with nonradioactive detection procedures.

Specific delineation of individual chromosomes by *in situ* hybridization (12–15), also termed chromosome painting, has been very useful for cytogenetic analyses in diagnostics and research. Further refinement of these approaches will require that probe sets for specific chromosomal subregions be generated (e.g., by DNA libraries from hybrid cell lines or microdissected chromosomes). The IRS-PCR products, as shown in the present study, provide an alternative approach to generate probe sets for delineation of entire chromosomes or chromosomal subregions. In contrast to the other procedures, no cloning protocols are required and only minimal preparation of probe DNA is needed.

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