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Original investigations

Painting of human chromosomes with probes generated from hybrid cell lines by PCR with Alu and L1 primers

C. Lengauer[1], H. Riethman[2], and T. Cremer[1]

[1] Institut für Humangenetik und Anthropologie der Universität, Im Neuenheimer Feld 328, W-6900 Heidelberg, Federal Republic of Germany
[2] Department of Genetics, Washington University, School of Medicine, St. Louis, MO 63110, USA

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Summary. Specific amplification of human sequences of up to several kb length has recently been accomplished in man-hamster and man-mouse somatic hybrid cell DNA by IRS-PCR (interspersed repetitive sequence – polymerase chain reaction). This approach is based on oligonucleotide primers that anneal specifically to human Alu- or L1-sequences and allows the amplification of any human sequences located between adequately spaced, inverted Alu- or L1-blocks. Here, we demonstrate that probe pools generated from two somatic hybrid cell lines by Alu- and L1-PCR can be used for chromosome painting in normal human lymphocyte metaphase spreads by chromosomal in situ suppression (CISS-) hybridization. The painted chromosomes and chromosome subregions directly represent the content of normal and deleted human chromosomes in the two somatic hybrid cell lines. The combination of IRS-PCR and CISS-hybridization will facilitate and improve the cytogenetic analysis of somatic hybrid cell panels, in particular, in cases where structurally aberrant human chromosomes or human chromosome segments involved in interspecies translocations cannot be unequivocally identified by classical banding techniques. Moreover, this new approach will help to generate probe pools for the specific delineation of human chromosome subregions for use in cytogenetic diagnostics and research without the necessity of cloning.

Introduction

Panels of interspecies hybrid cell lines, such as man-mouse or man-Chinese hamster cell lines, have provided an important tool for gene mapping. The usefulness of such panels for the mapping of human genes depends on the precise characterization of their human chromosome content. The human chromosomes in hybrid cell lines often undergo a variety of changes during construction and propagation of the somatic hybrids, including deletions, duplications, and interspecies translocations or insertions. Classical cytogenetic analyses of these hybrids are tedious and difficult, whereas analyses based on Southern hybridization are representative only for small chromosome regions around the mapped probe. Nonradioactive in situ hybridizaton (NISH) of total genomic human DNA has provided a tool for the specific delineation of human chromosome material in hybrid cell lines (Schardin et al. 1985; Manuelidis 1985; Durnam et al. 1985), but these analyses, even when combined with additional banding procedures, are hampered by the fact that the chromosomal origin of small centric fragments or small interspecies translocations and insertions still cannot be pinpointed unequivocally in many cases (A. Brückner and T. Cremer, unpublished observations).

Recently, chromosomal in situ suppression (CISS-) hybridization protocols have been developed that allow the use of complex probe sets, such as complete DNA libraries derived from sorted human chromosomes, to visualize individual chromosomes in a highly specific manner (Pinkel et al. 1988; Lichter et al. 1988a; Cremer et al. 1988). This technique, which is also referred to as chromosome painting (Pinkel et al. 1988), has provided a novel approach to improve the cytogenetic evaluation of hybrid cell lines (Kievits et al. 1990; Boyle et al. 1990). In this way, chromosomes or chromosomal subregions of a parental species present in a given hybrid cell line can be directly visualized in normal metaphase spreads of the parental species in question. For this purpose, Kievits et al. (1990) have preannealed biotinylated total man-mouse hybrid cell DNA with a titrated amount of unlabeled human genomic competitor DNA and hybridized the preannealed probe to normal human metaphase spreads. Similarly, Boyle et al. (1990) hybridized biotinylated total DNA of a mouse-hamster hybrid cell line to normal mouse metaphase spreads after preannealing with unlabeled genomic mouse and hamster competitor DNA. Since the chromosomes of interest present in these hybrid cell lines constitute only a minor fraction of the biotinylated total DNA, this elegant approach

Offprint requests to: T. Cremer

may have limited sensitivity (Boyle et al. 1990). In particular, chromosomes or chromosome segments that are only present in a small subpopulation of the hybrid cells may escape detection.

To increase further the sensitivity of this approach, we have applied IRS-PCR (interspersed repetitive sequence-polymerase chain reaction) (Nelson et al. 1989; Ledbetter et al. 1990). IRS-PCR allows the specific amplification of human DNA sequences from man-mouse or man-hamster hybrid cell DNA. For this purpose, oligonucleotide primers have been established that under appropriate conditions of stringency hybridize specifically to human Alu repeats or human L1 repeats but not to related sequences present in mouse or Chinese hamster genomic DNA. Alu-repeats and L1-repeats represent short and long interspersed repeat elements (SINES and LINES), respectively, which are present in approximately 10^6 (Alu) and 10^4–10^5 (L1) copies in the human genome. IRS-PCR of hybrid cell DNA with either the Alu primer 517 or the L1 primer recently described by Nelson et al. (1989) and Ledbetter et al. (1990) results in the amplification of any human DNA sequence located between two inverted blocks of human Alu- or L1-repeats, provided that these blocks are located within a distance that can be bridged by PCR.

As a model case, we have amplified human DNA sequences from the Chinese hamster hybrid cell line Cl 21 containing an apparently normal X chromosome as the only known human chromosome material, and from the mouse hybrid cell line RuRag 6 containing various human chromosomes in a small fraction of the cells. The amplification products were biotinylated and used as probes for chromosomal in situ suppression (CISS-) hybridization to metaphase spreads from normal PHA-stimulated human lymphocytes (Lichter et al. 1988a; Pinkel et al. 1988).

Materials and methods

Cell lines

The man-hamster hybrid cell line Cl 21, kindly provided by Dr. Peter Vogt, was originally established in Dr. Peter Goodfellow's laboratory (Imperial Cancer Research Fund Laboratories, London, UK). The man-mouse hybrid cell line RuRag 6 was kindly provided by Dr. K.-H. Grzeschik (Institute of Human Genetics, University of Marburg, FRG).

Chromosome preparations

Metaphase chromosome spreads were obtained from hybrid cell lines and from phytohemagglutinin (PHA)-stimulated normal male and female human blood lymphocytes using standard procedures (Cremer et al. 1988). Preparations were stored in 70% ethanol at 4°C until use.

DNA preparations

Human genomic DNA was prepared from blood, whereas mouse genomic DNA and Chinese hamster genomic DNA were prepared from liver (Maniatis et al. 1982). Recombinant bacteriophage DNA libraries from sorted human chromosomes were obtained from the American type culture collection (chromosome 7, LA07NS01; chromosome 11, LL11NS01; chromosome 20, LL20NS01; chromosome X, LA0XNL01). Libraries were amplified in liquid culture using *E. coli* LE 392 as the bacterial host. Purification of phages and extraction of phage DNA was carried out as described by Maniatis et al. (1982).

Interspersed repetitive sequence – polymerase chain reaction (IRS-PCR)

Hybrid cell DNA (100 ng) was used in a 100 µl assay containing 1 µ*M* Alu-primer 517 (Nelson et al. 1989) or L1-primer (Ledbetter et al. 1990), 10 m*M* TRIS-HCl (pH 8.4), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin, 250 µ*M* of each of the four dNTPs and 2.5 units of *Thermus aquaticus* DNA polymerase (Perkin Elmer/Cetus). As a control, the same assays were performed with mouse and Chinese hamster genomic DNA. Alu-PCR reaction conditions were as follows: after initial denaturation at 92°C for 3 min, 30 cycles of PCR were carried out with denaturation at 92°C for 1 min annealing at 61°C for 1 min, and extension at 72°C for 4 min. For L1-PCR reactions, the same conditions were used except that the annealing temperature was 55°C. PCR-products were ethanol precipitated and the pellets resuspended in double distilled water and analyzed by agarose gel electrophoresis.

CISS-hybridization and probe detection

Labeling of DNA probes with biotin-11 dUTP, CISS-hybridization and probe detection with avidin conjugated with fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC) was carried out as described previously (Lichter et al. 1988a) with the following modifications: 300 ng of biotinylated Alu- or L1-PCR-amplified hybrid cell DNA was used as a probe after preannealing with various amounts (3–9 µg) of an unlabeled Cot1-DNA fraction (BRL/Life Technologies, cat. no. 5279SA), kindly provided by Dr. Peter Lichter. The signals were amplified once according to the protocol of Pinkel et al. (1986). In double CISS-hybridization experiments, DNA probes labeled with digoxigenin (Kessler et al. 1990; Seibl et al. 1990) were applied accordingly. FITC-conjugated sheep-anti-digoxigenin Fab fragments (Boehringer Mannheim) were used for detection (Lichter et al. 1990). Chromosome preparations were counterstained with 0.2 µg/ml DAPI and 1 µg/ml propidium iodide, mounted in fluorescence antifading buffer (1 mg p-phenylendiamine in 1 ml glycerine buffer, pH 8.0), and evaluated with a Zeiss photomicroscope III equipped for epifluorescence. Microphotographs were taken with AGFACHROM 1000 RS color slide film.

Results

Optimization of IRS-PCR conditions

Annealing temperatures for the Alu- and L1-primers were systematically varied (data not shown) and chosen as low as possible to achieve primer annealing to consensus sequences of as many human Alu- and L1-repeat blocks as possible, but to avoid annealing to related sequences in the Chinese hamster and mouse genomes (see Methods for the protocol that yielded optimum results in our hands). Lower annealing and extension temperatures also resulted in substantial amplification of DNA sequences from these species (data not shown). Figure 1 shows IRS-PCR amplification products from the hybrid cell line Cl 21 and RuRag 6 after separation on a 1.2% agarose gel. Alu- and L1-PCRs reproducibly yielded distinctly different patterns of amplification products in both hybrid cell lines. The products ranged in size from approximately 300 bp up to 4 kb under our synthesis conditions. In contrast, no amplification products

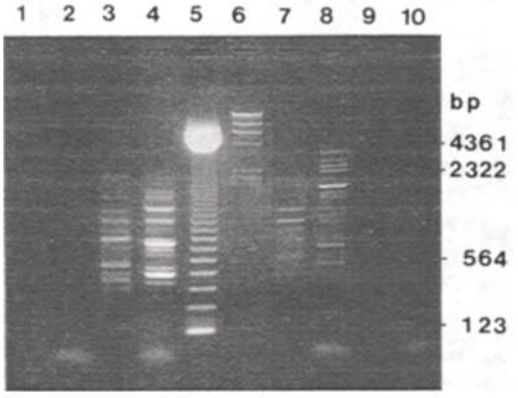

Fig. 1. Agarose gel electrophoresis of amplification products obtained by Alu-PCR *(lanes 1, 3, 7, 9)* and L1-PCR *(lanes 2, 4, 8, 10)* of various DNA sources. *Lanes 1, 2* Chinese hamster DNA (CHO) (negative control); *lanes 3, 4* man-Chinese hamster hybrid cell line Cl 21; *lane 5* 123 bp ladder (Bethesda Research Laboratories); *lane 6* lambda DNA digested with *Hin*dIII; *lanes 7, 8* man-mouse hybrid cell line RuRag 6; *lanes 9, 10* mouse liver DNA (negative control)

were seen when mouse or Chinese hamster genomic DNA was used as a template.

CISS-hybridization of IRS-PCR probes to human lymphocyte metaphase spreads

Results of typical CISS-hybridization experiments performed with biotinylated IRS-PCR amplification products as probes to normal human lymphocyte metaphase spreads are shown in Fig. 2a–h. Both Alu- and L1-PCR probes obtained from Cl 21 resulted in the exclusive painting of the whole X-chromosome (Fig. 2a, b). Painting of this chromosome was particularly intense when the two types of probes were applied jointly (Fig. 2c). These results are in agreement with previous cytogenetic analyses of Cl 21, which indicated that the X chromosome was the only human chromosome in this hybrid cell line.

Figure 2d shows a typical result of CISS-hybridization of the L1-amplification probe obtained from the RuRag 6 hybrid cell line to a normal human male metaphase spread. Both chromosomes 11 are painted along their whole length. In contrast, the single human X chromosome is specifically covered with fluorescent dots only on its long arm. Similarly, the long arm is exclusively painted in the two chromosomes 7, although painting of this arm is more intense. Figure 2 (f, g) demonstrates painted chromosomes 7 at higher magnification. In addition, slight painting of chromosome 20 was also occasionally observed (not shown). Control experiments using identical amounts of total hybrid cell DNA instead of the IRS-PCR probes did not result in any detectable signal (not shown).

CISS-hybridization of human chromosomes in RuRag 6 hybrid cells

The experiments described above indicate the presence of human chromosomes 11, 20, 7q and Xq in RuRag 6 hybrid cells. The following experiments were performed to determine the percentage of hybrid cells containing these chromosomes and chromosomal subregions. For this purpose, bacteriophage DNA libraries from the respective chromosomes were hybridized under suppression conditions to hybrid cell metaphase spreads and interphase nuclei. These experiments confirmed the presence of human chromosomes 7, 11, 20 and X in this cell line (Table 1a, b). Whereas chromosomes 11 and 20 appeared structurally normal in most metaphase spreads, chromosomes 7 and X showed deletions of the short arm. Interspecies translocations were also seen in rare cases (not shown). Notably, fluorescent in situ hybridization with biotinylated human genomic DNA showed the presence of human chromosome material only in a minor fraction of metaphase spreads and interphase nuclei (Table 1a, b). In most cases, the labeled cells contained only one labeled human metaphase chromosome or interphase chromosome domain. Percentages of interphase nuclei with painted human chromosome domains were lower than the percentages of painted metaphase spreads (compare Table 1a with b). We suggest that this difference indicates that probe penetration in interphase nuclei was less effective than in metaphase chromosomes.

The results so far do not exclude the possibility that a small fraction of RuRag 6 hybrid cells contains human chromosome material other than the material described above. To solve this question, a double fluorescent in situ hybridization experiment was carried out. In this experiment, human genomic DNA, labeled with digoxigenin, was cohybridized to hybrid cells with biotinylated, combined bacteriophage DNA from sorted human chromosomes 7, 11, 20, and X libraries. After detection of the digoxigenin-labeled human genomic sequences with FITC and of the biotinylated sequences with TRITC, all analyzed cells, which showed FITC-signals indicative for the presence of human chromosome material (10 metaphase spreads and 240 interphase nuclei), also showed colocalization of the TRITC-signal (Fig. 2i–k). We conclude from this result that the human chromosome material that can be detected in the RuRag 6 hybrid cell line by in situ hybridization with human genomic DNA is indeed fully represented by chromosomes 7, 11, 20 and X as described above.

Discussion

The results of this study demonstrate that IRS-PCR with human SINES and LINES directed oligonucleotide primers (Nelson et al. 1989; Ledbetter et al. 1990) can be used to generate probes from somatic hybrid cell lines that are useful for the painting of specific human chromosomes and chromosomal subregions. This approach provides a new and rapid strategy for the cytogenetic characterization of somatic hybrid cell panels used for human gene mapping studies, and is sensitive enough to detect human chromosomes present in only a minor fraction of the cells in a hybrid cell line. The following steps are performed sequentially. First, IRS-PCR sequences are generated from the hybrid cell line under investigation. CISS-hybridization of this probe to normal human

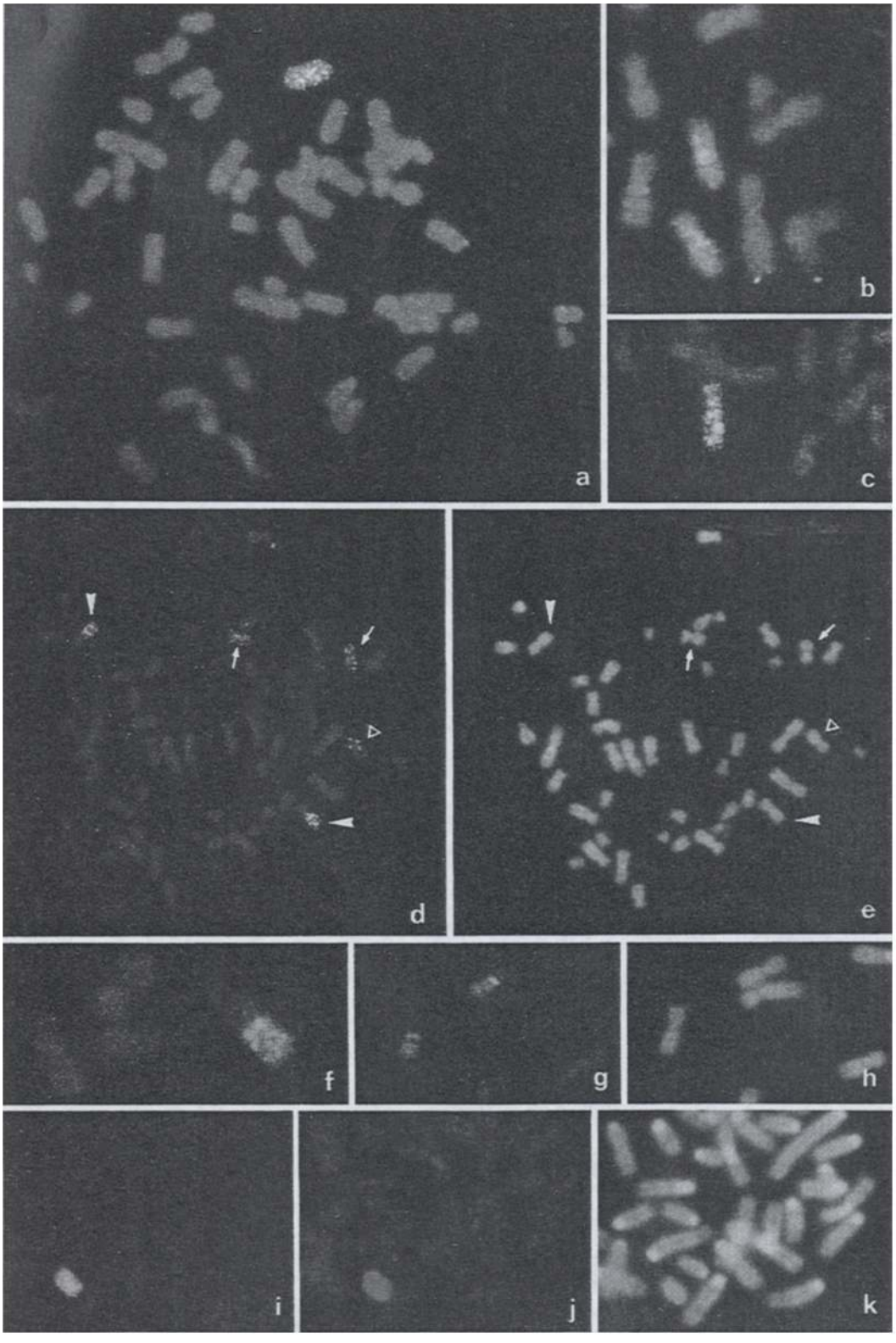

Fig. 2. a–h CISS-hybridization of biotinylated IRS-PCR amplified probes produced from somatic hybrid cell lines Cl 21 or RuRag 6 to metaphase spreads from normal male and female PHA-stimulated lymphocytes. Probes were detected with avidin-FITC (**a–d, f, g**) and chromosomes counterstained with propidiumiodide (**a–d, f**) and/or DAPI (**e, h**). **a** Painting of the X-chromosome in a normal male human metaphase spread by CISS-hybridization with Alu-PCR amplified Cl 21 DNA. **b** Painting of the two X-chromosomes in a normal female human metaphase spread by CISS-hybridization with L1-PCR amplified Cl 21 DNA. **c** Painting of the X-chromosome in a normal male metaphase spread by CISS-hybridization with pooled Alu-PCR and L1-PCR amplified Cl 21 DNA. **d** Painting of chromosome 11 *(arrows)*, the long arm of chromosomes 7 *(arrowheads)* and the long arm of the X chromosome *(open triangle)* in a normal male metaphase spread by CISS-hybridization with L1-PCR amplified RuRag 6 DNA reflects the presence of the respective human chromosome material in the RuRag 6 hybrid cell line. **e** The same metaphase spread as in **d**

Table 1. Human chromosomes painted in metaphase spreads and interphase nuclei of the man-mouse hybrid cell line RuRag 6 by fluorescent in situ hybridization with biotinylated human genomic DNA or by CISS-hybridization with biotinylated phage DNA libraries from sorted human chromosomes 7, 11, 20 and X. Percentages of RuRag 6 metaphase spreads exhibiting 0, 1, 2, 3 and more painted normal or abnormal human chromosomes are indicated on the left, and percentages of RuRag 6 interphase nuclei exhibiting 0, 1, 2, 3 or more distinctly painted human chromosome territories on the right

a Metaphase spreads ($n \sim 50$)					**b** Interphase nuclei ($n = 1000$)				
Chromosome	Signal (%)				Chromosome	Signal (%)			
	0	1	2	≥3		0	1	2	≥3
7	82	16	2	–	7	92.5	6.5	0.9	0.1
11	88	12	–	–	11	93.8	5.8	0.4	–
20	89.5	10.5	–	–	20	94.7	5.1	0.2	–
X	95	5	–	–	X	95.2	4.6	0.2	–
Human genomic DNA	71	26	3	–	Human genomic DNA	83.0	14.6	2.4	–

chromosome complements is performed to determine human chromosomes and chromosome fragments contained in the particular cell line. Step 1 will be very useful in the identification of human chromosome material in cases where routine banding analysis does not provide definitive results. Secondly, CISS-hybridization using libraries from sorted human chromosomes or pools of subregional probes selected on the basis of the results achieved in step 1 can provide direct evidence of the presence and frequency of specific human chromosomes or chromosome segments in metaphase spreads and interphase nuclei of the investigated hybrid cell line. Thirdly, a pool of bacteriophage DNA libraries from the identified human chromosomes, modified with one non-radioactive label (e.g., biotin), can be cohybridized with total genomic human DNA, chemically modified in a different way (e.g., with digoxigenin). The differently labeled sequences can then be visualized with different fluorochromes (e.g., FITC [green] and TRITC [red]). The colocalization of green and red signals in all hybrid cell metaphase spreads and interphase nuclei containing human chromosome material suggests that the hybrid cell line does not contain additional unidentified human chromosome material. Although small interspecies translocations and insertions may still have escaped detection, cytogenetic evaluation may be considered complete for most practical purposes at this stage of the analysis.

The advantages and limitations of IRS-PCR amplification of human sequences as a tool for the correct cytogenetic analysis of hybrid cells need to be investigated further. The results obtained in this study with the RuRag 6 hybrid cell line provide a case in point. Although painting of the long arm of chromosome 7 and of whole chromosomes 11 was easily recognized, painting of the long arm of the X-chromosome was weak and painting of chromosome 20 was borderline. The extent of human sequence amplification that can be obtained by IRS-PCR for an individual human chromosome or chromosome fragment contained in a somatic hybrid cell line probably depends on several factors, such as the PCR conditions, the number and distribution of chromosome-specific amplifiable regions, the fraction of cells containing the particular chromosome material, and the presence of other human chromosomes that compete for oligonucleotide primers. The possibility that some of the IRS-PCR-generated sequences may crosshybridize to non-targeted chromosomes also has to be taken into consideration. All of these factors will influence the sensitivity and accuracy with which normal metaphase spreads painted with the amplified sequences mirror the chromosomes and chromosomal fragments present in a tested hybrid cell line.

SINES have been shown to cluster preferentially in Giemsa light bands, whereas LINES cluster in Giemsa dark bands (Korenberg and Rykowski 1988; Chen and Manuelidis 1989). The use of primers directed to both short (Alu) and long (L1) interspersed repeat elements was therefore expected to increase the coverage of a given human chromosome with amplified sequences. In this study, we have applied each primer in separate PCR-assays. Combination of the amplification products from each assay produced particularly strong signals on the painted chromosomes. Alternatively, combination of the two primers in a single PCR-assay should result in additional amplified sequences that could not be generated by either primer alone (Ledbetter et al. 1990). The power of this technique as a tool for chromosome painting may be further increased, if oligonucleotide primers specific for other interspersed repetitive sequences are included in these assays.

IRS-PCR of hybrid cell lines containing deleted human chromosomes, interspecies translocations or insertions of interest, should also become a useful and simple adjunct to other approaches that are available to generate probes for the selective painting of chromosomal subregions, such as microlibraries from microdissected chromosomes (Lengauer et al. 1990), YAC-clones (Wada et al. 1990) or band-specific pools of phage or cosmid DNA clones (Lichter et al. 1988b; Riethman et al. 1989). These approaches will fill the gap between the selective painting of complete human chromosomes with DNA libraries from whole sorted chromosomes (see Introduction) and painting of specific genes with individual DNA clones at all stages of the cell cycle (e.g., Landegent et al. 1987; Garson et al. 1987; Bhatt et al. 1988; Albertson et

counterstained with DAPI. **f** Normal chromosome 7 painted with L1-PCR amplified RuRag 6 DNA at higher magnification. **g** Two normal chromosomes 7 painted with Alu-PCR amplified RuRag 6 DNA. **h** Same chromosomes as shown in **g** counterstained with DAPI. **i–k** Double fluorescence in situ hybridization with human genomic DNA (labeled with digoxigenin and detected with FITC) and pooled phage DNA libraries from sorted human chromosomes 7, 11, 20, X (biotinylated and detected with TRITC) to a RuRag 6 metaphase spread. Chromosome painted with FITC (**i**) demonstrating its human origin is also delineated with TRITC (**j**) indicating human chromosome 7, 11, 20 or X material. **k** Same chromosomes as shown in **i**, **j** counterstained with DAPI

al. 1988; Lawrence et al. 1988; Trask et al. 1989; Lichter et al. 1990; Ried et al. 1990). These applications taken together will have an important impact in all areas of human cytogenetics (for review see Lichter and Ward 1990). Finally, it should be noted that IRS-PCR in conjunction with oligonucleotide primers specific for interspersed sequences of other species and appropriate somatic cell hybrids may also be used to generate probes for chromosome painting in these other species.

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References

Albertson DG, Fishpool R, Sherrington P, Nacheva E, Milstein C (1988) Sensitive and high resolution in situ hybridization to human chromosomes using biotin labelled probes: assignment of human thymocyte CD1 genes to chromosome 1. EMBO J 7:2801–2805

Bhatt B, Burns J, Flannery D, McGee O'D (1988) Direct visualization of single copy genes on banded metaphase chromosomes by non-isotopic in situ hybridization. Nucleic Acids Res 16:3951–3961

Boyle AL, Lichter P, Ward DC (1990) Rapid analysis of mouse-hamster hybrid cell lines by in situ hybridization. Genomics 7:127–130

Chen TL, Manuelidis L (1989) SINEs and LINEs cluster in distinct DNA fragments of Giemsa band size. Chromosoma 98:309–316

Cremer T, Lichter P, Borden J, Ward DC (1988) Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome-specific library probes. Hum Genet 80:235–246

Durnam DM, Gelinas RE, Myerson D (1985) Detection of species specific chromosomes in somatic cell hybrids. Somat Cell Mol Genet 11:571–577

Garson JA, Berghe JA van den, Kemshead JT (1987) Non isotopic in situ hybridization technique detects small (1kb) unique sequences in routinely G-banded human chromosomes: fine mapping of N-myc and β-NGF genes. Nucleic Acids Res 15: 4761–4770

Kessler C, Höltke HJ, Seibl R, Burg J, Mühlegger K (1990) Non-isotopic labeling and detection of nucleic acids. I. A novel DNA labeling and detection system based on digoxigenin: antidigoxigenin ELISA principle (digoxigenin system). Biol Chem Hoppe-Seyler (in press)

Kievits T, Devilee P, Wiegant J, Wapenaar MC, Cornelisse CJ, Ommen GJB van, Pearson PL (1990) Direct nonradioactive in situ hybridization of somatic cell hybrid DNA to human lymphocyte chromosomes. Cytometry 11:105–109

Korenberg JR, Rykowski MC (1988) Human genome organization: Alu, Lines, and the molecular structure of metaphase chromosome bands. Cell 53:391–400

Landegent JE, Jansen in de Wal N, Dirks RW, Baas F, Ploeg M van der (1987) Use of whole cosmid clones genomic sequences for chromosomal localization by non-radioactive in situ hybridization. Hum Genet 77:366–370

Lawrence JB, Villnave CA, Singer RH (1988) Sensitive, high resolution chromatin and chromosome mapping in situ: presence and orientation of two closely integrated copies of EBV in a lymphoma line. Cell 52:51–61

Ledbetter SA, Nelson DL, Warren ST, Ledbetter DH (1990) Rapid isolation of DNA probes within specific chromosome regions by interspersed repetitive sequence (IRS) PCR. Genomics 6:475–481

Lengauer C, Eckelt A, Weith A, Endlich N, Ponelies N, Lichter P, Greulich KO, Cremer T (1990) Painting of defined chromosomal regions by in situ suppression hybridization of libraries from laser-microdissected chromosomes. Cytogenet Cell Genet (in press)

Lichter P, Ward DC (1990) Is non-isotopic in situ hybridization finally coming of age? Nature 345:93–95

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC (1988a) Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using recombinant DNA libraries. Hum Genet 80:224–234

Lichter P, Cremer T, Tang CC, Watkins PC, Manuelidis L, Ward DC (1988b) Rapid detection of chromosome 21 aberrations by in situ hybridization. Proc Natl Acad Sci USA 85:9664–9668

Lichter P, Tang CC, Call C, Hermanson G, Evans GA, Housman D, Ward DC (1990) High-resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. Science 247:64–69

Maniatis T, Fritsch EF, Sambrook J (eds) (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Manuelidis L (1985) Individual interphase chromosome domains revealed by in situ hybridization. Hum Genet 71:288–293

Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis R, Webster TD, Ledbetter DH, Caskey CT (1989) Alu polymerase chain reaction: a method for rapid isolation of human-specific sequences from complex DNA sources. Proc Natl Acad Sci USA 86:6686–6690

Pinkel D, Gray JW, Trask B, Engh G van den, Fuscoe J, Dekken H van (1986) Cytogenetic analysis by in situ hybridization with fluorescently labeled nucleic acid probes. Cold Spring Harb Symp Quant Biol 51:151–157

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray JW (1988) Fluorescence in situ hybridization with human chromosome specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85: 9138–9142

Ried T, Mahler V, Vogt P, Blonden L, Ommen GJB van, Cremer T, Cremer M (1990) Direct carrier detection by in situ suppression hybridization with cosmid clones of the Duchenne/Becker muscular dystrophy locus. Hum Genet 85:581–586

Riethman HC, Moyzis RK, Meyne J, Burke DT, Olson MV (1989) Cloning human telomeric DNA fragments into *Saccharomyces cerevisiae* using a yeast-artificial-chromosome vector. Proc Natl Acad Sci USA 86:6240–6244

Schardin M, Cremer T, Hager HD, Lang M (1985) Specific staining of human chromosomes in Chinese hamster × man hybrid cell lines. Hum Genet 71:281–287

Seibl R, Höltke HJ, Rüger R, Meindl A, Zachau HG, Raßhofer R, Roggendorf M, Wolf H, Arnold N, Wienberg J, Kessler C (1990) Non-radioactive labeling and detection of nucleic acids. III. Applications of the digoxigenin system. Biol Chem Hoppe Seyler (in press)

Trask B, Pinkel D, Engh G van den (1989) The proximity of DNA sequences in interphase cell nuclei is correlated to genomic distance and permits ordering of cosmids spanning 250 kilobase pairs. Genomics 5:710–717

Wada M, Little RD, Abidi F, Porta G, Labella T, Cooper T, Della Valle G, D'Urso M, Schlessinger D (1990) Human Xq24–Xq28: approaches to mapping with yeast artificial chromosomes. Am J Hum Genet 46:95–106