Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources

(somatic cell hybrid/X chromosome/cloning/yeast artificial chromosome)

DAVID L. NELSON*†‡, SUSAN A. LEDBETTER‡, LAURA CORBO‡, MAUREEN F. VICTORIA‡,
RAMIRO RAMÍREZ-SOLIS‡, THOMAS D. WEBSTER‡, DAVID H. LEDBETTER‡, AND C. THOMAS CASKEY*‡

*Howard Hughes Medical Institute and ‡Institute for Molecular Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

ABSTRACT   Current efforts to map the human genome are focused on individual chromosomes or smaller regions and frequently rely on the use of somatic cell hybrids. We report the application of the polymerase chain reaction to direct amplification of human DNA from hybrid cells containing regions of the human genome in rodent cell backgrounds using primers directed to the human *Alu* repeat element. We demonstrate *Alu*-directed amplification of a fragment of the human *HPRT* gene from both hybrid cell and cloned DNA and identify through sequence analysis the *Alu* repeats involved in this amplification. We also demonstrate the application of this technique to identify the chromosomal locations of large fragments of the human X chromosome cloned in a yeast artificial chromosome and the general applicability of the method to the preparation of DNA probes from cloned human sequences. The technique allows rapid gene mapping and provides a simple method for the isolation and analysis of specific chromosomal regions.

Somatic cell hybrids containing human chromosomes or subchromosomal fragments isolated in rodent cell backgrounds have served as powerful tools for gene mapping as well as for the isolation of chromosome-specific sequences (1). Isolation of human sequences from hybrid cells requires the production of a recombinant library and the identification of clones based upon hybridization to human repetitive sequences (2). To isolate a large number of human clones from a region, a large library must be constructed and screened. We endeavored to develop a technique that would circumvent construction of libraries from hybrids and thus allow the isolation of human-specific sequences directly from the hybrid DNA. This has led to a general strategy for the rapid preparation of human-specific DNA from a variety of sources, including sequences in yeast artificial chromosome (YAC) (3) and bacteriophage vectors.

Mammalian genomes contain short interspersed repeat DNAs (SINES) (4); in man, the major family of this type is denoted the *Alu* repeat sequence (5). These repeats are found ubiquitously in human DNA and are believed to number 900,000 in the haploid human genome, giving an average distance between copies of ≈4 kilobases (6). This distance may vary considerably, since *Alu* sequences appear to be enriched in certain chromosomal regions and deficient in others (7). Repetitive elements homologous to the human *Alu* repeat are also found in rodent genomes. However, there is sufficient sequence divergence to reduce cross hybridization of human and rodent *Alu* repeats. We have made use of this difference to allow direct amplification of human sequences specifically from hybrid cells in mouse and hamster back-

grounds through the use of the polymerase chain reaction (PCR).

MATERIALS AND METHODS

Cell Lines. The somatic cell hybrids used in this study are described in Table 1. The hybrid lines 94-3, 8121 Aza 1, and 2384 Aza 2 were derived by fusing a V79 hamster cell line with lymphoblastoid cell lines of patients with X chromosome deletions or translocations (12) and will be described elsewhere. The line Micro 28g contains a fragile X chromosome broken at the fragile (X) site and translocated to hamster (13).

PCRs and Primers. The PCR was carried out in a total volume of 100 μl with 1 μg of DNA, primer at 1 μM in 50 mM KCl/10 mM Tris·HCl, pH 8.0/1.5 mM MgCl$_2$/0.01% gelatin/ 300 μM dATP/300 μM dCTP/300 μM dGTP/300 μM dTTP (Pharmacia), and 2.5 units of *Thermus aquaticus* polymerase (Perkin–Elmer/Cetus) for 35 cycles of 94°C denaturation (1 min), 55°C annealing (45 sec), and 68°C extension (5 min) in an automated thermal cycler (Perkin–Elmer/Cetus). Initial denaturation was 4 min at 94°C. Variations from these reaction conditions: Primer 517 was used at 0.1 μM; in Fig. 3B each dNTP was used at 200 μM. Primers for the PCR were synthesized on an Applied Biosystems DNA synthesizer (model 380B) and were used in reactions after deprotection without further purification. PCR primer sequences are as follows: TC-65, AAGTCGCGGCCGCTTGCAGTGAGC-CGAGAT; 278, CCGAATTCGCCTCCCAAAGTGCTGG-GATTACAG; 32, ACTCGGGAGGCTGAGGCAGG; 33, CTCGGCTCACTGCAAACTCC; 34, ATCGCATGAAC-CCGGGAGGC; 515, GCATCGATAGATYRYRCCAYTG-CACT; 517, CGACCTCGAGATCTYRGCTCACTGCAA; T7, CGAATGCGGCCGCTAATACGACTCACTATAGGG; T3, ATTAACCCTCACTAAAGGGA; where Y is a pyrimidine and R is a purine.

DNAs and Vectors. The following probes were used: HPRT cDNA [a 731-base-pair (bp) fragment of the cDNA pHPT31 (16)] factor VIII cDNA [114.12, a 650-bp genomic fragment containing exons 17 and 18 (17)], G6PD cDNA [GDP 25a, a 1.5-kilobase (kb) partial cDNA (18)], color blindness [hs7, a 1.2-kb cDNA (19)], and total human DNA (placenta). Vectors used were λDASH (Stratagene) and pYAC4 (3).

YACs. Clones were generated by partial *Eco*RI digestion of high molecular weight DNA from the X3000-11.1 hybrid and ligation with the pYAC4 vector that had been digested with *Eco*RI and *Hind*III and dephosphorylated as described (3). The eight clones described were selected on the basis of hybridization to total human DNA.

Gel Electrophoresis and Southern Hybridization. DNAs were visualized with ethidium bromide after electrophoresis in 1.1% agarose gels. Size markers are a mixture of λDNA

Abbreviations: PCR, polymerase chain reaction; YAC, yeast artificial chromosome; APF, *Alu* PCR fragment.
†To whom reprint requests should be addressed.

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Table 1. Human chromosome complements of somatic cell hybrids used in these studies

Cell line	Background	X chromosome region	Other human chromosome(s)	Ref.
4.12	Hamster	Entire X	None	8
X3000-11.1	Hamster	Xq24-qter	None	9
F649-5	Hamster	Xq25-qter	der 3, 8, 11	10
RJK 734	Mouse	Xq26-qter	11pter-q23	11
94-3	Hamster	Xq26-qter	15pter-q25, 10, 22	12
8121 Aza 1	Hamster	Xpter-q26.3	6,7,14,21	12
2384 Aza 2	Hamster	Xpter-q27.1	6,11,17p-,21	12
Micro 28g	Hamster	Xpter-q27.3	None	13
MH22-6	Mouse	None	17	14
HD1132b	Mouse	None	4	15

digested with *Hind*III and ΦX174 DNA digested with *Hae* III. Southern transfers to nylon membranes (GeneScreen*Plus*, NEN/DuPont) were done by standard protocols (20). DNA probes were labeled with ^{32}P by the random-hexamer method (21). Hybridization was carried out in 1% SDS/1 M NaCl/ 10% (wt/vol) dextran sulfate with herring sperm or human placental carrier DNA (500 μg/ml). Posthybridization washes were 2× SSC/0.1% SDS at room temperature, 2× SSC/0.1% SDS 65°C, and 0.1× SSC/0.1% SDS 65°C (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0).

RESULTS

Human-Specific Primers. Fig. 1A shows the consensus *Alu* repeat sequence and the locations of the primers we constructed. Our initial criteria for design of primers were to utilize regions of the *Alu* repeat that would be highly conserved among copies of the repeat found in the GenBank DNA resource (release 60.0) and located close to one of the ends of the consensus sequence. Primer TC-65 includes a 13-base 5′ extension containing a *Not* I cloning site and 17 bases of *Alu* sequence at the 3′ end, and primer 278 was constructed with 25 bases of *Alu* sequence and 8 bp of 5′ extension containing an *Eco*RI site.

Each primer was used individually in a PCR with the expectation that sequences between two *Alu* repeats in inverted orientation relative to one another could be amplified (Fig. 1B). Amplification with primer TC-65 generated a smear of sequences from human DNA, a larger number of individual bands from hybrid DNAs, and no amplification

from hamster or mouse DNA (Fig. 2A). Primer 278 generated smears of amplified sequence from all DNA sources (data not shown). Comparison of the *Alu* consensus sequence from human and hamster revealed that primer 278 was directed to a region of the repeat that is highly conserved between rodents and primates, and primer TC-65 was located within a 31-bp sequence that is unique to the second monomer of primate *Alu* repeats (5). In addition, oligonucleotides 32, 33, 34, and 515 (Fig. 1A) show results in hamster DNA similar to those observed with primer 278. A primer that recognizes the same 17 bp of *Alu* sequence but in the opposite orientation (primer 517) also serves to amplify human DNA specifically (Fig. 2B).

By using primers TC-65 and 517, amplification with a number of somatic cell hybrids retaining various amounts of the human X chromosome along with various other human chromosomes in rodent cell backgrounds was attempted (Fig. 2). There does not appear to be a strict correlation between amount of human DNA contained in the hybrid and the number of bands amplified (e.g., compare MH22-6, a chromosome 17 hybrid, to HD1132b, a chromosome 4 hybrid), although a background smear of DNA in the lane is much more pronounced in hybrids with multiple human chromosomes. The fact that total human DNA amplifies as a smear suggests that sufficient numbers of fragments are amplified to obscure individual sequences.

Separate amplifications of different hybrid cell lines containing overlapping regions of the same human chromosome show similar patterns of bands. This is most readily apparent comparing hybrids 4.12, X3000-11.1, and Micro 28g in amplifications with primer 517 in Fig. 2B. Hybrid 4.12 carries an intact X chromosome, hybrid X3000-11.1 contains only an Xq24-qter chromosome, and hybrid Micro 28g contains an Xpter-q27 chromosome. *Alu* PCR fragments (APFs) found in hybrid X3000-11.1 are a subset of the APFs from hybrid 4.12, and the hybrid Micro 28g APFs are nearly identical to those in hybrid 4.12, with the exception of the bottom-most band at ≈500 bp. These results allow the regional assignment of the 500-bp band to Xq28 on the basis of its absence from hybrid Micro 28g. Confirmation of this localization has been obtained by using the 500-bp fragment to probe the amplified fragments from the other members of the mapping panel (data not shown).

***Alu* Amplification from the *HPRT* Gene.** To investigate the nature of the *Alu* PCR products, probes from the region of the X chromosome represented in the hybrids were used to
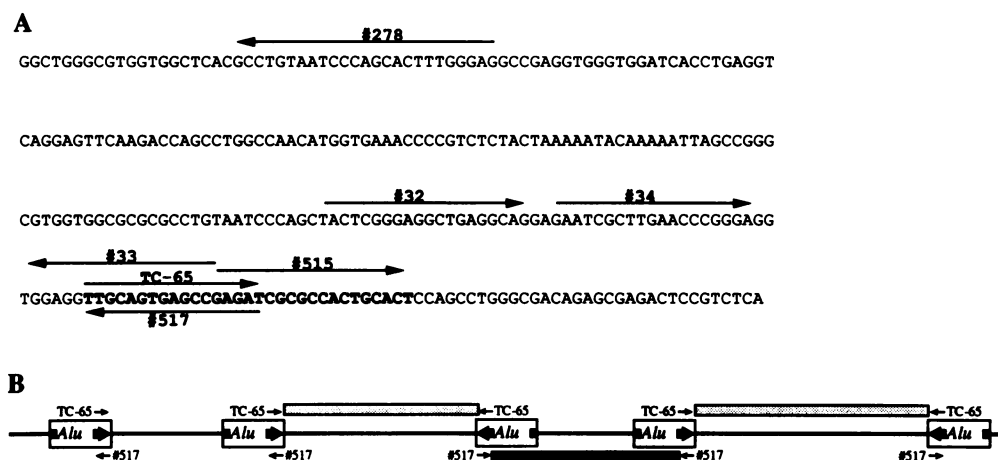
A

B

Fig. 1. Consensus *Alu* sequence and scheme for amplification. (*A*) Consensus *Alu* sequence and locations of primers for amplification. The sequence derived from Kariya *et al.* (22) is shown. The 31-bp region that is unique to the second monomer of primates is in bold type. PCR primers are shown as lines with arrowheads to indicate 5′ to 3′ orientation relative to the *Alu* sequence. Some primers contain 5′ extensions; the sequence of each is given in *Materials and Methods*. (*B*) Cartoon showing types of amplification expected with the human specific primers TC-65 and 517.
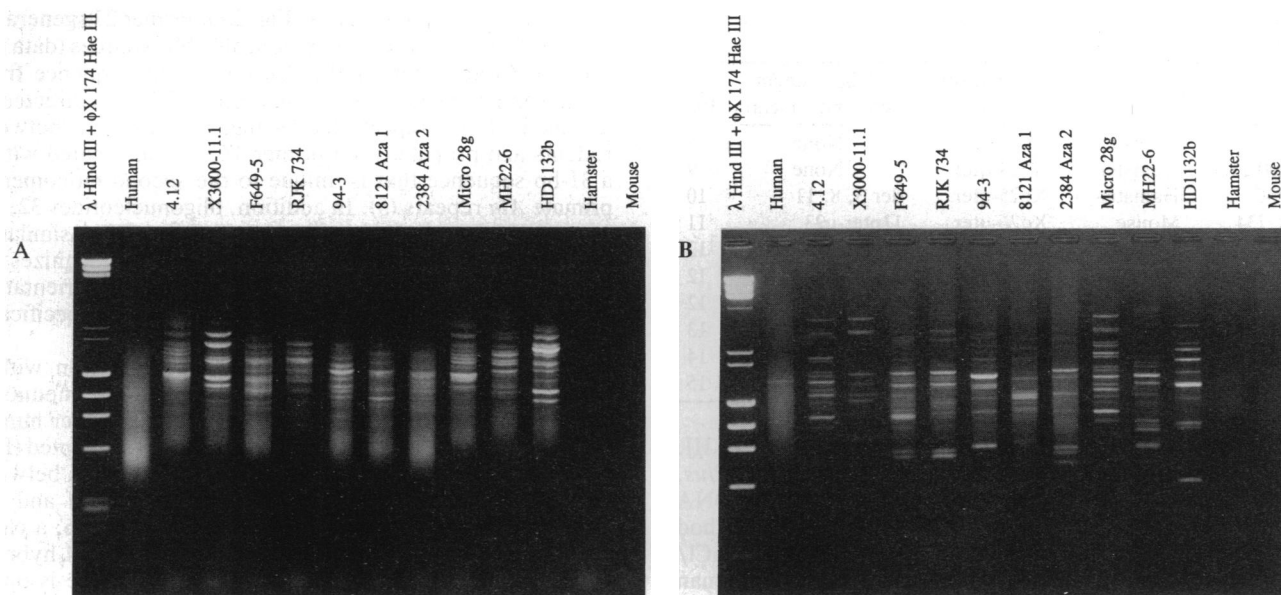
FIG. 2. Amplification of human DNA from somatic cell hybrids by using *Alu* primers. (*A*) Results of PCRs with primer TC-65 of DNA from human, mouse, and hamster as well as from 10 hybrid cell lines containing various human chromosomes (Table 1) and with X chromosome segments depicted in Fig. 4. (*B*) Results of PCRs using primer 517 for the same DNA samples as in *A*.

determine if known regions were present. A band of ≈3.5 kb is found in amplification with primer TC-65 when hybridized to an HPRT cDNA probe (16). This band was present in amplifications from all hybrids containing the gene but varied in intensity depending upon the amount of human sequence in the hybrid (data not shown). Three probes for other genes in this region of the X chromosome (clotting factor VIII, color blindness, and G6PD) showed no hybridization to Southern blots of *Alu* PCR products with primer TC-65.

Hybridization of this product to the HPRT cDNA probe must result from amplification of sequences containing one or more exons of the *HPRT* gene. We identified candidate exons using six genomic λ clones derived from and completely overlapping the *HPRT* locus (Fig. 3*A*) (23, 24). Fig. 3*B* shows amplified sequences derived from the six clones using primer TC-65 as well as DNA from hybrid X3000-11.1. Two of the clones clearly demonstrate a band of the appropriate size, and this band is positive for hybridization with the HPRT cDNA probe (Fig. 3*C*). The band comigrates with the HPRT-positive band found in amplification of hybrid X3000-11.1. These two clones contain only the last three exons of the gene (exons 7, 8, and 9). Clone Huλ14, which also contains the final three exons, shows no amplification, allowing placement of one of the *Alu* repeats 3' to the end of this clone.

Analysis of sequence data derived from the λ clones composing the locus (A. Edwards, W. Ansorge, and C.T.C., unpublished data) allowed identification of the *Alu* repeats involved in this amplification. The *Alu* repeats are the appropriate distance apart (3.5 kb), are in opposite orientation, allowing amplification with primer TC-65, and span exon 9 (the largest *HPRT* exon). Sequence of the repeats surrounding the primer binding site is shown in Fig. 3*D*. The 5' *Alu* sequence (*Alu* A) contains a single mismatch in the primer-binding region, whereas the 3' repeat (*Alu* B) is perfectly matched. *Alu* A shows an additional 2-base match of the first two nucleotides of the tail of the primer; this may contribute to the favored amplification of this sequence and compensate for the mismatch in the *Alu* portion. No other significant homology between the 5' tail of the primer and the *Alu* sequence is found.

Alu **PCR for Isolation of Inserts in YAC Clones.** YAC cloning allows isolation of extremely large inserts (up to 10^6 bp) (3). Application of the *Alu* PCR to human DNA cloned in

YAC vectors allows a rapid means of isolating insert fragments for further analysis. The *Alu* PCR of 1 μg of total yeast DNA from eight human YAC clones (average insert size, 150 kb) derived from hybrid X3000-11.1 with primer 517 provided single bands from two of the human clones. Through the use of additional *Alu* primers and primers directed to pYAC4 sequences adjacent to the insert, we have amplified sequence from all eight clones.

Fig. 4 shows hybridization of a ^{32}P-labeled 1100-bp primer-517-primed *Alu* PCR product from YAC clone X2-327 to hybrid DNAs constituting a subchromosomal mapping panel for the terminal end of human chromosome Xq. Hybridization was carried out in an excess of human placental DNA to block hybridization of the *Alu* repeats present on the PCR product. The pattern of hybridization allows an assignment of this YAC clone to Xq24-25. *Alu* PCR products from seven YACs used as probes in this manner have allowed a map position to be determined; the eighth contained too much repetitive sequence to be unambiguously assigned (data not shown).

Probe Preparation from Clones in Bacteriophage Vectors. The capability of YAC inserts to amplify with *Alu* primers led us to investigate the feasibility of this approach for rapid isolation of insert sequences from clones with small inserts. To apply the *Alu* PCR to a group of clones in the λDASH vector, we designed primers homologous to the T7 and T3 bacteriophage RNA promoter sequences adjacent to the cloning site of the vector, allowing the combination of *Alu* and vector primers for the PCR. Amplification is obtained from all clones when either the T3 or the T7 primer is used in combination with primer TC-65 (data not shown). The starting material for amplification can be phage DNA, lysate, or primary plaque isolates. Probes derived from these products yield identical map assignments as those derived from the entire phage DNA, indicating that the products are human specific and derived from the clone (data not shown).

DISCUSSION

Demonstration of human-specific amplification using oligonucleotide primers directed to repetitive sequences provides the opportunity for rapid isolation and analysis of small regions of human DNA in complex backgrounds. This
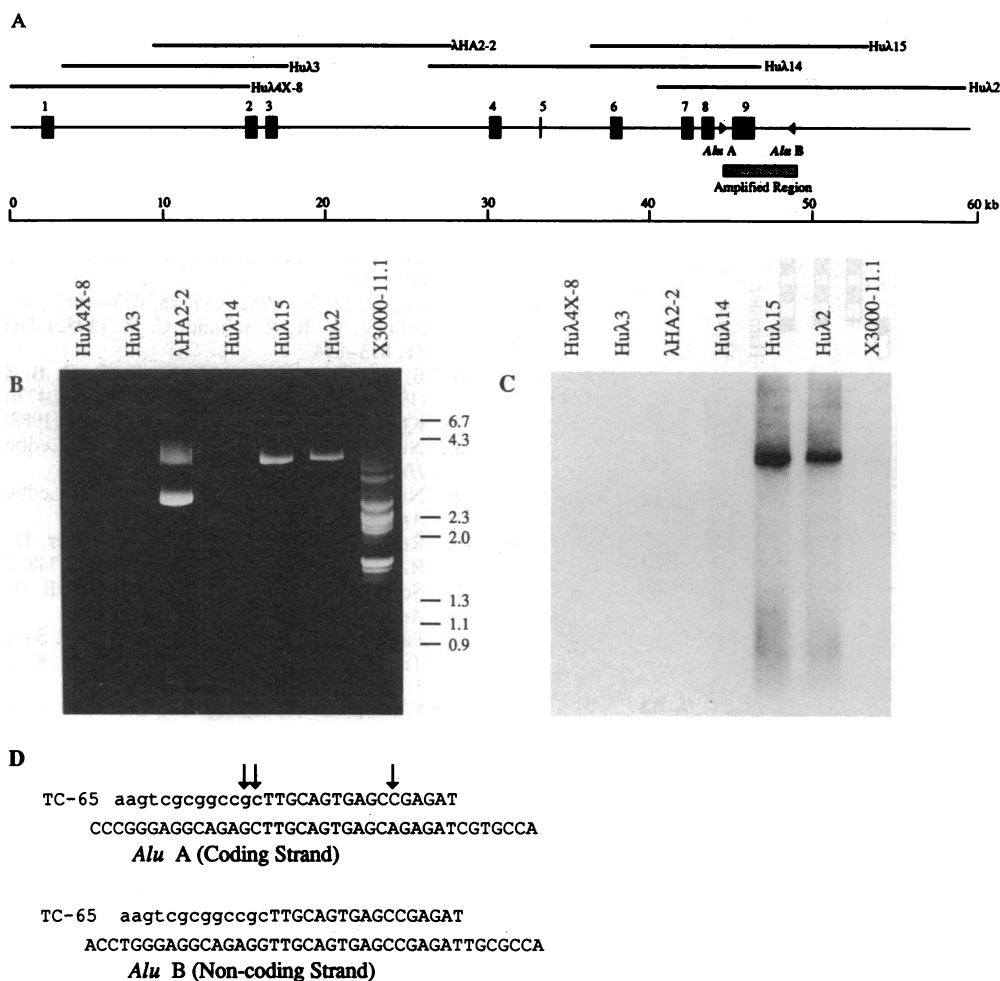
FIG. 3.   Amplification of a segment of the human *HPRT* locus with *Alu* primer TC-65. (*A*) Scale map of the *HPRT* locus showing the positions of each of the nine exons (boxes), the regions covered by each of six bacteriophage inserts (lines above) encompassing the gene, and the positions of the *Alu* A and B sequences involved in amplification with primer TC-65. (*B*) Ethidium bromide-stained gel of TC-65 PCR products from six HPRT bacteriophage clones and DNA from hybrid X3000-11.1. (*C*) Autoradiogram of the gel in *B* after Southern hybridization to the HPRT cDNA probe. Exposure is overnight without intensifying screen. (*D*) Forty base pairs of sequence of the *Alu* repeats involved in TC-65 amplification in the TC-65 homologous region. Sequence is derived from the bacteriophage clone HuλI5. Arrows (*Alu* A sequence) indicate a match of the first two nucleotides of primer TC-65 "tail" with the next two 5' bases of the *Alu* sequence and a mismatch between the *Alu* homologous portion of TC-65 and this *Alu* repeat.

method can be used for both analytical and preparative purposes. It is currently capable of providing material from human chromosomes in hybrid cells as well as from clones in YAC and λ vectors and can allow characterization of sequences present in such complex DNA mixes.

The human-specific primers described (primers TC-65 and 517) amplify a small fraction of the sequences present between *Alu* repeats in the somatic cell hybrids used. For primer TC-65, we estimate a fragment is produced for each 500 kb of human DNA. Primer 517 produces a fragment every 1000 kb. Only selected pairs of *Alu* repeats are providing amplification with these primers and conditions. Analysis of the *Alu* repeats involved in the PCR from the *HPRT* locus allows the determination that complete sequence homology with the primer is not necessary for the favored amplification of this sequence from somatic cell hybrid DNA. Likewise, extensive homology to the 5' extension contained in the primer is not an important parameter.

It is possible that a majority of *Alu* repeats have diverged significantly in the 17-bp sequence used as a primer and that only a small number of *Alu* pairs is sufficiently homologous to allow amplification. However, analysis of a large number of *Alu* repeats from GenBank shows this sequence to be quite well conserved, and of 20 *Alu* repeats found in the *HPRT*

locus, 7 show complete matches for this 17-bp region whereas each of the remaining 13 contains a single mismatch. Mismatches are known to be allowed in PCR amplification (see above and ref. 25) but are not favored when alternative primers are present (26). In this case, alternative templates are available to a constant primer sequence.

It may be that the bias is due to pairs of *Alu* repeats occurring in the same orientation preferentially. It is worth noting that for the *HPRT* locus pairs of *Alu* repeats are found largely in the same orientation. The sequence of the plasminogen activator inhibitor 1 gene contains 12 *Alu* repeats, all of which are in the same orientation, on the mRNA-coding strand (27). This may represent a general phenomenon (28) that could explain the relative lack of amplified sequence from large regions of the genome with single *Alu* primers.

The *Alu* PCR applied to YAC and λ clones allows rapid isolation of insert sequences for analysis. In YACs, this is preferable to the alternative of gel isolation of the artificial chromosome (29). Probes derived from a vector–*Alu* PCR should be valuable to efforts to "chromosome walk" (30, 31). We have observed specific patterns of bands with *Alu* amplification in YAC clones. As with the somatic cell hybrids described above, these APFs should allow the use of the *Alu* PCR analytically as a rapid alternative to the current "fin-
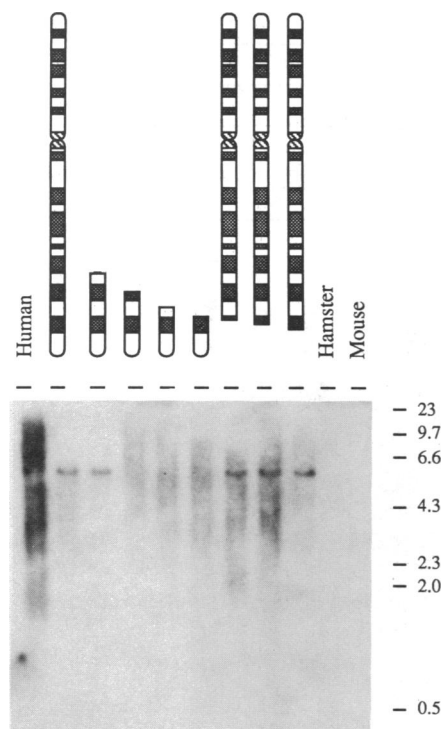
FIG. 4. Use of *Alu* PCR products as probes to determine the chromosomal location of the YAC insert. A 1300-bp band from YAC X2-327 amplified with primer 517 was ^{32}P-labeled and hybridized to a Southern blot of *Eco*RI-digested DNAs from human, hamster, mouse, and eight somatic cell hybrids. The order of the hybrid cell lines (from left to right) is as follows: 4.12, X3000-11.1, F649-5, RJK 734, 94-3, 8121 Aza 1, 2384 Aza 2, and Micro 28g. The hybridization utilized human placental DNA as carrier DNA to eliminate hybridization of human repetitive sequences. The blot was exposed for 6 days with an intensifying screen at −70°C.

gerprinting" methods for identification of potentially overlapping sequences among inserts in YAC and other cloning vectors.

Future Prospects. Modifications that allow amplification of the majority of *Alu* sequences from a region or clone [either through the use of primers that provide bidirectional amplification from a single *Alu* repeat using two primers or by inverted PCR (32)] will further enhance the utility of the technique. In the ideal case, this would allow the direct creation of probes from regions retained in somatic cell hybrids for use in screening recombinant libraries derived from total human DNA, obviating the need for creation of a recombinant DNA library from a hybrid cell line containing the region of interest. The use of the *Alu* PCR for probe preparation should greatly enhance the capacity for characterization of cloned fragments of human chromosomes by eliminating DNA preparation and insert purification. The *Alu* PCR and its variants should become important methods for reducing the complexity and increasing the speed of genome analysis.

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