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Polymerase chain reactions with alphoid-repeat primers in combination with *Alu* or LINEs primers, generate chromosome-specific DNA fragments

R. P. ERICKSON,[1,2]* T. W. GLOVER,[1,2] B. K. HALL[1] AND M. WITT[2]†

[1] *Departments of Pediatrics and Communicable Diseases and* [2] *Human Genetics, D1109 MPB, Box 0718, University of Michigan School of Medicine, Ann Arbor, MI 48109-0178*

SUMMARY

Y alphoid primers in combination with *Alu* and LINEs primers generated new DNA fragments in polymerase chain reactions (PCR) on DNA from a Y-only somatic cell hybrid but not from X-only, 3-only, or 21-only hybrids. X alphoid primers used in a similar manner generated new DNA fragments from the X-only hybrid, and 1 of the primers (X_2) also generated new DNA fragments on 3-only and 21-only hybrids when used in conjunction with *Alu* or LINEs primers. In all but one case, consensus alphoid primers generated new chromosome-specific fragments in PCR reactions with the *Alu* or LINEs primers. A search for cryptic *Alu*- or alphoid-alone PCR products as the source for one *Alu*-alphoid band (chosen at random) was negative. Partial sequencing of products demonstrated that alphoid and *Alu* sequences were indeed contiguous in some newly synthesized DNA fragments. While *Alu* or LINEs primers generate smears of DNA fragments on total human DNA, the alphoid-nonalphoid repeat combinations generated electrophoretically distinguishable bands of DNA when the template was total DNA. While these were distinguishable with different chromosome-specific alphoid primers, the DNA fragments were not of the same sizes as those generated with the chromosome-only hybrids.

INTRODUCTION

Recently Nelson *et al.* (1989) introduced a novel use of the polymerase chain reaction (PCR) to rapidly amplify and isolate human DNA from complex sources. Knowing that the genome has about 900000 copies of the *Alu* repeat, they used a single primer designed to hybridize to *Alu* sequences and performed PCR on somatic cell hybrids with reduced amounts of human DNA or on YAC clones with fragments of human DNA. While PCR with a single *Alu* primer generated a smear of DNA fragments when total human DNA was used as template, copies of *Alu* were oriented towards each other and were close enough to each other to generate single bands from hybrids containing the X chromosome or subportions of the X chromosome (Nelson *et al.* 1989). Ledbetter *et al.* (1990) extended the technique by using single primers to the long interspersed repeat element (LINEs) either alone or in combination with the *Alu* primers. They found that the combination of *Alu* and LINEs primers generated new bands which were not

* To whom correspondence should be addressed at present address: Department of Pediatrics, University of Arizona, Health Sciences Center, Tucson, Arizona 85724, U.S.A.

† Present address: Institute of Human Genetics, Polish Academy of Sciences, Strzeszynska 32, 60–479 Pozan, Poland.

detected with either primer alone. We have extended this approach to combinations of alphoid, centromeric repeat primers and *Alu* or LINEs primers in order to generate further new markers for specific chromosomes as well as to try to isolate pericentromeric sequences.

MATERIALS AND METHODS

PCR Primers

Y_1, Y_2 primers, flanking the 170 bp fragment of the alphoid repeats of the human Y chromosome, and X_1, X_2 primers flanking the 130 bp fragment of the alphoid repeats of the human X chromosome, have previously been described (Witt & Erickson, 1989) but the correct Y_1 sequence is ATGATAGAAACGGAAATATG (a 1 bp deletion was published). A pair of 5′ (CCTTCGTTGGAAACGGGATT) and 3′ (AAAAGAGGTCTGAATATACCA) primers, based on a consensus alphoid repeat, were also synthesized using the sequence reported by Wu & Manuelidis (1980). As an *Alu* primer, we used the 517 primer sequence reported by Nelson *et al.* (1989) while the LINEs primer sequence used was LIHs from Ledbetter *et al.* (1990). Oligodeoxynucleotides were synthesized on an Applied Biosystem 380A DNA synthesizer and were not purified by HPLC.

Cell Lines

3E7 contains the Y chromosome as its only human element on a mouse background (Goodfellow *et al.* 1983) and was the gift of Peter Goodfellow. FUDR 1·6 contains most of the human X; from Xp telomere → Xq27 on a Chinese hamster background (Glover & Stein, 1988). UCTP-2A is a hybrid containing only human chromosome 3 in a Chinese hamster cell line deficient for UMP synthetase activity (Patterson *et al.* 1983) and was a gift from Charles Scoggins. The WA17 somatic cell hybrid contains human chromosome 21 on a mouse background (Raziuddin *et al.* 1984). DNA from this cell line was the gift of Maggie Van Kueren and David Kurnit.

Polymerase chain reactions and electrophoresis of products

Polymerase chain reactions were carried out in the presence of 10 mM Tris-HCl, pH 7·6, 50 mM KCl, 1·5 mM $MgCl_2$, 0·01 % gelatin, 200 mM dNTP's, 1 mM primers (except primer 517 which was 0·1 mM), 2·5 units Taq polymerase and 100 ng template DNA in a 100 μl reaction volume. After addition of 50 μl mineral oil to prevent evaporation, the samples were amplified on a Coy Laboratories thermal cycler (model 50). Initially the samples were denatured at 94 °C for 5 minutes, annealed at 55 °C for 45 seconds and extended at 68 °C for 4 minutes for 1 cycle. This was followed by 34 cycles at 94 °C for 1 minute, 55 °C for 45 seconds and 68 °C for 4 minutes. Following amplification, 50 μl chloroform and 10 μl 6X tracking dye were added to the samples which were then vortexed and centrifuged briefly. Fifty μl of the sample were then loaded into a 1·5 % low melting point agarose gel in 1 X TBE (Maniatis *et al.* 1982) containing 4 μg/ml ethidium bromide and electrophoresed at 40 volts for 16 hours. Size markers were a mixture of DNA cut with *Hin*dIII and ϕX174 DNA cut with *Hae*III. PCR products were visualized on a 300 nm UV light box and photographed with a polaroid camera.

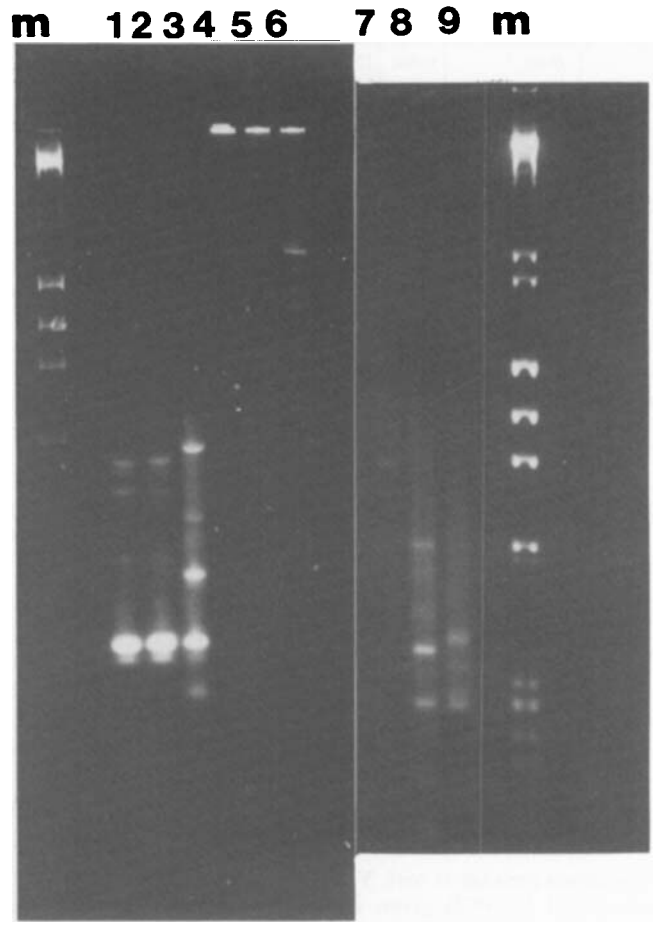

Fig. 1. Agarose gel electrophoresis of PCR reactions with LINEs or *Alu* and Y_1 or Y_2 primers on 3E7 or mouse DNA. Lanes 1–3 and 7–9, 3E7, a Y-only hybrid; lanes 4–6, total mouse DNA. Primers used in lanes: (1) LIHs; (2) LIHs and Y_1; (3) LIHs and Y_2; (4)–(6) as 1–3; (7) 517; (8) 517 and Y_1; (9) 517 and Y_2. M = markers of DNA cut with *Hin*dIII and ϕ X174 DNA cut with *Hae*III.

Sequencing

The DNA fragments of 355 bp and 281 bp generated from the 3E7, Y-only hybrid with the 517 *Alu* and Y_1, alphoid primers were cut out of the gel and eluted by soaking in 10 mM Tris, 1 mM EDTA, pH 7·4, for several days. DNA was re-amplified with the same PCR primers and an aliquot examined on agarose gel electrophoresis. The final pure PCR product was precipitated and dissolved in a small amount of water. The DNA was then sequenced directly using the dideoxy-termination method and reagents as provided in the Sequenase T7 DNA Polymerase Kit (U.S. Biochem. Corp.). The U.S. Biochem. Corp. procedure for sequencing with PCR was used. The samples were electrophoresed in a 6·0% 7 M urea sequencing gel, fixed, dried, and exposed to X-ray film (Kodak XAR-5) for 2–7 days.

cell lines	517	LIHs	Y_1	Y_2	Y_1-517	Y_2-517	Y_1-LIHs	Y_2-LIHs
mouse	none	none	none	1500, 1100, 330	none	none	none	(1500,1100), 680
hamster	none	none	none	1500,1350, 1000,900, 872,650, 520,310, 230	none	as Y_2 with some bands gone	none	as Y_2 with some bands gone
3E7 (Y only on mouse)	870,1150, 1400	550,530, 480,330, 180,170	none	1080,680, 660,650, 510,500, 330,310, 85	**603, 355, 281**	**380,330, 281**	no Δ from LIHs alone	**590,450, 300,100,** (180,170)
FUDR 1.6 (X only on hamster)	3>resolution 2150,1800	1550,1500 1200,1100 520,500, 290	none	no Δ from hamster alone	no Δ from 517 alone	subset of bands of Y_2 on hamster	no Δ from LIHs alone	no Δ from LIHs alone
UCTP-2A (3 only on hamster)	3050,3000, 1450,1150	1800,1300, 1150,650, 580,500,480, 315,250	none	no Δ from hamster alone	none	smear with bands as in Y_2 on hamster	no Δ from LIHs alone	no signif Δ from LIHs alone
WA17 (21 only on mouse)	2600,2300, 2250	3500,2600, 2000,1350, 1300,1280, 900,700, 600,230	none	2400, 1400 1200, 1000 610, 570, 500, 330	**2400, 1200 950, 900, 800, 610, 500, 480, 330**	(1400, 1000) **780, 620, 560, (500) 450, 350**	**3000,** (2600) **2400,** (1350) **1200, 1150** (900), **590, 220**	(2400) **1020 490, 400, 240**
Total human (♂) DNA	none	none	none	1450,1250, 1150,1080	none	none	**440, 420**	**1200, 1070 440, 220**

Fig. 2. Sizes of PCR fragments generated with Y_1 or Y_2 primers and *Alu* or LIHs primers. Band sizes of DNA fragments generated by PCR given in bp. Fragments seen in appropriate controls are bracketed while newly generated, specific fragments are highlighted.

RESULTS

Alphoid, non-alphoid repeat PCR reactions generate new chromosome-specific DNA fragments

The chromosome-specific alphoid primers, in PCR reactions with repeat primers, generally generated new DNA fragments identifiable as prominent, discrete bands after electrophoresis in agarose (other, fainter, new bands were less reproducible). Using the Y-only hybrid, 3E7, *Alu* primer 517 together with alphoid primer Y_1 or Y_2 generated such bands while the LINEs primer LIHs plus Y_2 generated novel bands (Figs. 1 and 2). The Y_2 primer alone, and in combination with LIHs, generated some bands on the mouse only background (Figs. 1 and 8). Most of the reactions have been performed at least two times with nearly identical results. However, in other replications, changes in batches of enzyme, sources of water, or other as yet unidentified variables have sometimes resulted in fewer or greater numbers of these prominent DNA fragments. In the case of the Y_1, Y_2 primers and *Alu* or LINEs, the new fragment sizes were quite small, ranging in size from 100 to 600 bp. We were concerned that such small PCR fragments might arise from within the alphoid blocks of repeats themselves since the 2·4 kb, *Hph*I, Y chromosomal repeat contains an *Alu* element (Frommer *et al.* 1984). Thus, we

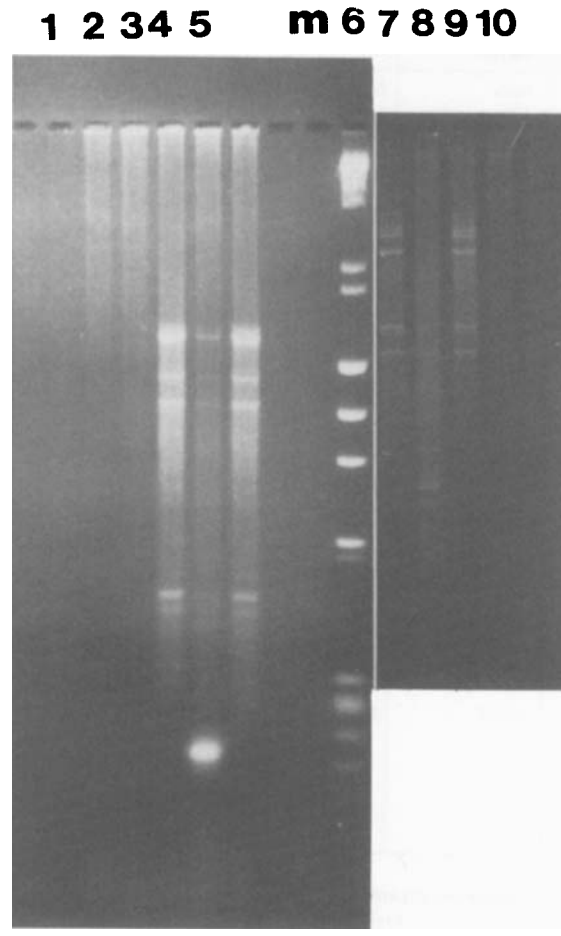

Fig. 3. Agarose gel electrophoresis of PCR reactions with LINEs or *Alu* and X_1 or X_2 primers on FUDR 1·6 DNA, and X-only hybrid. Primers used in lanes: (1) and (10) X_1 alone; (2) and (9) X_2 alone; (3) LIHs; (4) LIHs and X_1; (5) LIHs and X_2; (6) 517 alone; (7) 517 and X_2; (8) 517 and X_1. M = markers as in Fig. 1 for lanes 1–5. Lanes 6–10 are photographed at the same magnification but the gel had not been run as long as for lanes 1–5; fragment sizes given in Fig. 4 were derived from other markers not shown.

performed the same PCR reactions on 5 ng of Y97, a Y cosmid containing repeats of the 5·5 kb, *Eco*RI alphoid block (Wolfe *et al.* 1985). No bands were seen.

Using the X-only hybrid, new DNA fragments were generated by *Alu* primer 517 together with the alphoid X_2 primer and by LIHs and X_1 (Figs. 3 and 4). In this case, the new fragment sizes ranged from 210 to 1350 base pairs. The 210 bp fragment generated with LIHs plus X_1 was particularly intense.

We tested the chromosomal specificity of these reactions by using the X and Y combinations of primers on, respectively, Y-only and X-only somatic cell hybrids and on 3-only and 21-only somatic cell hybrids (Figs. 2, 4 and 5). The Y_2 primer alone produced DNA fragments with both 3-only and X-only hybrids which were also found with hamster DNA alone (Fig. 5). No new fragments were seen in the LIHs combinations with Y primers on somatic cell hybrids containing other chromosomes; new bands seen with 517 and Y_2 were seen on hamster DNA alone. The X_2 primer generated new DNA fragments in combination with 517 or LIHs on 3-only

cell lines	517	LIHs	X_1	X_2	X_1-517	X_2-517	X_1-LIHs	X_2-LIHs
mouse	none	none	none	none	none	780	none	none
hamster	none	none	none	none	none	none	none	none
FUDR 1.6 (X only on hamster)	3>resolution 2150,1800	1500,1550 1200,1100 520,500, 290	none	none	no Δ from 517	(1800) **1350,1075, 880,800,620**	(1550,1500, 1200,1100, 520,500, 290)**210**	no Δ from LIHs alone
UCTP-2A (3 only on hamster)	3050,3000, 1450,1150	1800,1300, 1150,650, 580,500, 480,315, 250	none	faint 1800	none	**600,520, 400,380**	no Δ from LIHs alone	(1800,1300, 1150,650, 580,500,480, 315,250) **1070**
WA17 (21 only on mouse)	2600,2300, 2250	3500,2600, 2000,1350, 1300,1280, 900,700, 600,230	none	none	none	**1280,1200, 1100,900, 850,800,750, 680,600,500, 350,310**	no Δ from LIHs alone	LIHs bands + **600,590, 310,280, 230**
Total human DNA	none	none	none	1800 1400 950 650 200	**700**	none	**410, 360, 310, 255, 205**	**410, 360, 255, 235**

Fig. 4. Sizes of PCR fragments generated with X_1 or X_2 primers and *Alu* or LIHs primers. Otherwise as in Fig. 2.

and 21-only hybrids which were not seen on the respective rodent DNAs and were different in the two cases. Thus, the X_2 primer did not seem to be as chromosome specific.

Alphoid, non-alphoid repeat PCR reactions generate new DNA fragments from many chromosomes

Although X-specific (Yang *et al.* 1982) and Y-specific (Wolfe *et al.* 1985) alphoid sequences were readily identified, it has been more difficult to find chromosome-specific alphoid repeats for autosomes. Alphoid repeats showing preferential localization to human chromosomes 4 and 9 (Hulsebos *et al.* 1988), 5 and 19 (Hulsebos *et al.* 1988), 5 (Jabs *et al.* 1984), 7 (Waye *et al.* 1987), 12 (Baldini *et al.* 1990), 13 (Warren *et al.* 1990), 13, 18 and 21 (Devilee *et al.* 1986*a*), 15 (Greig *et al.* 1989), 17 (Waye & Willard, 1986), 18 (Devilee *et al.* 1986*b*), 21 (Jorgensen *et al.* 1987), and 22 (McDermid *et al.* 1986) have been reported. To determine the generality of our approach, we used consensus alphoid 5′ and 3′ primers in combination with *Alu* and LINEs primers on a number of single human chromosome hybrids (Fig. 6). Although these primers generated bands when used singly (Fig. 8), when used with *Alu* and LINEs primers, the 5′ and 3′ consensus generated new and different DNA fragments ranging in size from 118 to 1900 bp. The only case in which new fragments were not generated was the combination of the 5′ alphoid and LIHs when used with DNA from the 21-only hybrid.

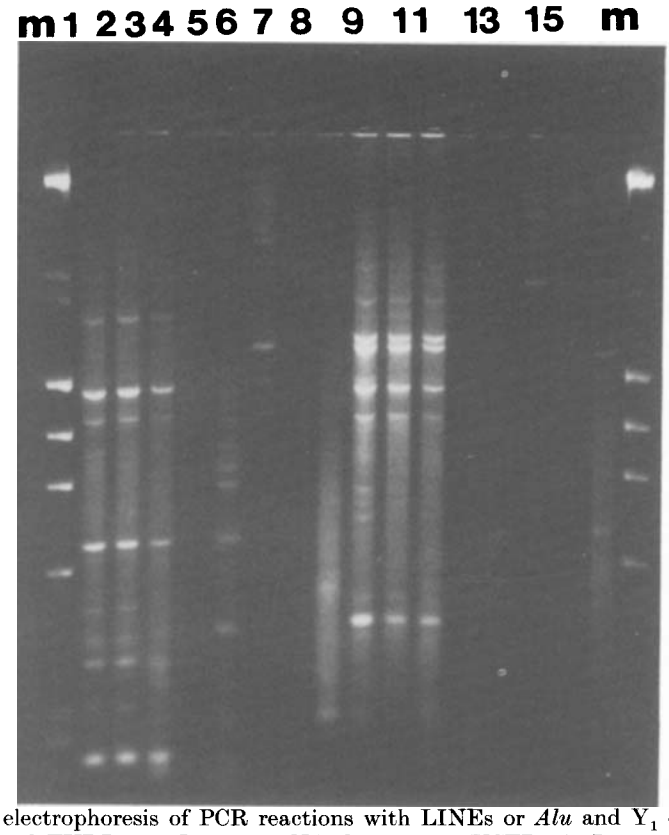

Fig. 5. Agarose gel electrophoresis of PCR reactions with LINEs or *Alu* and Y_1 or Y_2 primers on UCTP-2A (3-only) and FUDR 1·6 (X-only) DNA. Lanes 1–8, UCTP-2A. Lanes 9–16, FUDR 1·6. Primers used in lanes: (1) LIHs; (2) LIHs and Y_1; (3) LIHs and Y_2; (5) Y_2; (6) 517; (7) 517 and Y_1; (8) 517 and Y_2; (9)–(16) as in 1–8. M = markers as in Fig. 1.

cell lines	517 alone	LIHs alone	5'	3'	5'-517	3'-517	5'-LIHs	3'-LIHs
hamster	none	none	none	1400,1300, 960,900	none	none	2550	1400 960
mouse	none	none	1150	none	none	none	950,570, 420,250,70	none
UCTP-2A (3 only on hamster background)	3050,3000, 1450,1150	1800,1300, 1150,650, 580,500, 480,315, 250	1150	1500,1400 1100,1000	**1800 320**	**600** (+ smear)	**1900,**(1300) (1150)**870, 860,680,** (580,250)	**1900,**(1300) (1150)**680** (250)
WA17 (21 only on mouse background)	2600,2300, 2250	3500,2600, 2000,1350, 1300,1280, 900,700, 600,230	1150 480, 118	1500,1250, 830,	**490, 400, 120**	**870,860, 820,610, 600,500, 400,118**	as in LIH alone or mouse alone	(2500)(1400) (1300)**1100, 950,870** (600)**480, 420,320 200,118**

Fig. 6. Size of PCR fragments generated with consensus alphoid primers and *Alu* or LIHs primers. Otherwise as in Fig. 2.

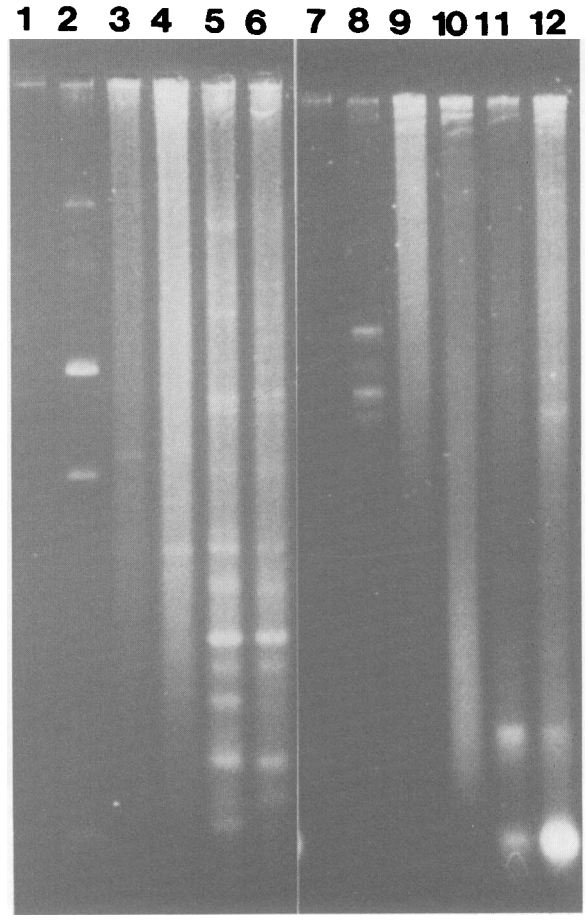

Fig. 7. Agarose gel electrophoresis of PCR reactions of alphoid repeat primers and *Alu* or LINEs primers on total human (male) DNA. Lanes: (1) X_1, (2) X_2; (3) X_1+517; (4) X_2+517; (5) X_1+LIHs; (6) X_2+LIHs; (7) Y_1; (8) Y_2; (9) Y_1+517; (10) Y_2+517; (11) Y_1+LIHs; (12) Y_2+LIHs.

Although the *Alu* (Nelson *et al.* 1989) and/or LINEs (Ledbetter *et al.* 1990) primers had generated electrophoretically-detected smears of DNA fragments when used alone on total human DNA, we tested the alphoid-*Alu* or LINEs primer combinations on total human DNA. As seen in Fig. 7 and summarized in Figs. 2 and 4, specific bands were clearly visible and were different for the Y alphoid combinations as compared with the X alphoid combinations. However, the bands were not the same ones as those which had been found on the particular chromosome-specific hybrids. It is likely that primer competition contributes to the appearance of unique-sized DNA fragments in these alphoid-repeat combination PCRs performed on total genomic human DNA but the source of the bands is not readily attributable to the particular chromosomes.

A search for 'cryptic' Alu- *or alphoid-alone bands as the source of* Alu-*alphoid bands*

Since there was some variability in the apparent quantity of newly generated *Alu*-alphoid PCR products (as visualized by ethiduim bromide staining after gel electrophoresis), the

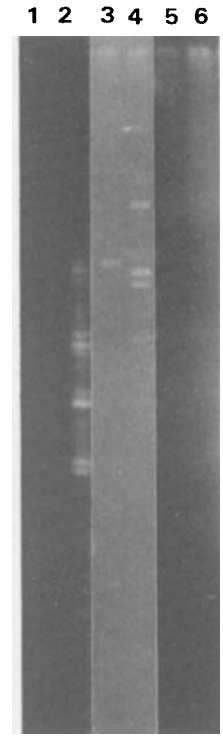

Fig. 8. Agarose gel electrophoresis of PCR reactions of alphoid repeat primers and 3′ alphoid consensus on UCTP-2A used individually. Lanes: (1) Y_1 on 3E7; (2) Y_2 on 3E7; (3) 5′ alphoid consensus on UCTP-2A; (4) 3′ alphoid consensus on UCTP-2A; (5) 5′ alphoid consensus on FUDR 1·6; (6) 3′ alphoid consensus on FUDR 1·6.

possibility that the 'new' bands were *Alu*- or alphoid-only primed products whose synthesis was increased by the presence of the other primer needed to be considered. We searched for 'cryptic' *Alu*- or alphoid-alone bands by performing a repeat PCR after gel electrophoresis of *Alu*- or alphoid-alone PCR products, using DNA recovered from the region of the gel where a new *Alu*-alphoid band was apparently detected. As seen in Fig. 9, PCR amplification of DNA eluted from 'plugs' of agarose gel picked up with a Pasteur pipette resulted in re-amplification of the 1800 bp band from the PCR products using 5′ consensus and 517 primers on UCTP-2A but not from the same region of the 5′ consensus- or 517-alone PCR products. A slightly smaller *Alu*-alone band is seen in all the PCR reactions where the 517 primer is present. This minor band, and smaller bands, are an indication of the sensitivity of the PCR reaction and the degree to which DNA fragments can be found anywhere between the well and their major location as a well defined band.

DNA sequencing confirms Alu, *alphoid repeat proximity*

In order to characterize the alphoid-repeat generated fragments in greater detail, partial sequencing was performed on re-amplified, eluted fragments using PCR primers as primers for dideoxy-sequencing. When the sequencing reactions were primed with the Y_1 alphoid repeat, in 2 cases *Alu* consensus sequences were detected (Table 1). In the case of 'band 2', a 281 bp fragment generated by PCR with Y_1 and 517 on 3E7 DNA, 47 base pairs of the 56 nucleotide

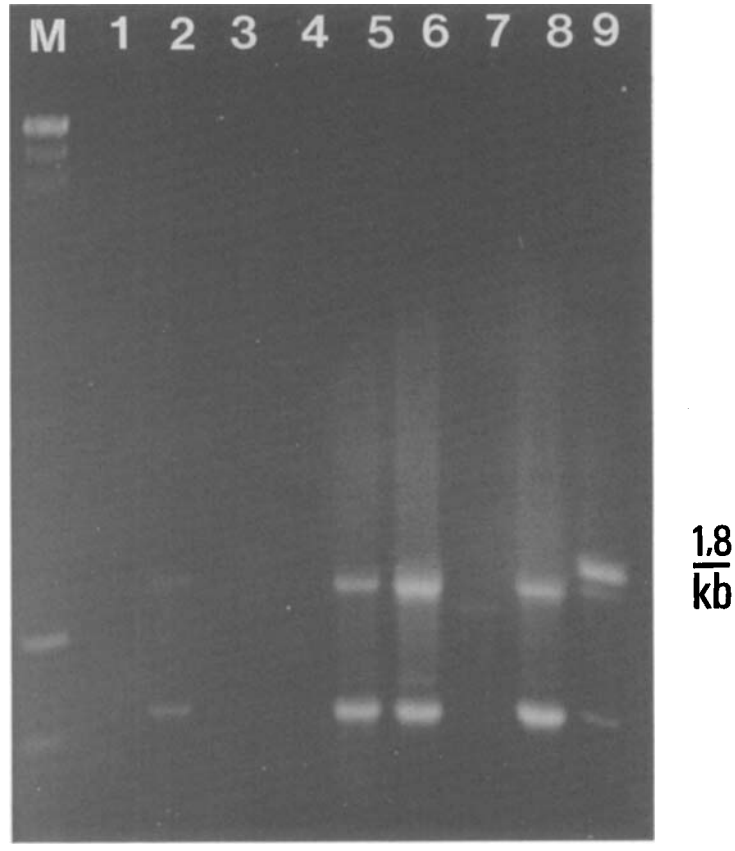

Fig. 9. Agarose gel electrophoresis of PCR reactions with 5′ consensus and/or *Alu* primers on DNA eluted from plugs of agarose taken from the 1800 bp positon on: lanes 1–3, 5′ consensus primed PCR; lanes 4–6, 517 primed PCR; lanes 7–9, 5′ consensus and 517 primed PCR. The PCR reactions were primed with: lanes 1, 4, 7–5′ consensus; lanes 2, 5, 8–517; and lanes 3, 6, 9–5′ consensus and 517. M = markers.

overlap were identical to an *Alu* consensus sequence. In the case of 'band 1A', a 355 bp fragment also generated by PCR with Y_1 and 517 on 3E7 DNA, the homology to the consensus *Alu* sequence was only 54% in the expected orientation but 84% to the reverse orientation, while a search of EMBL 19 detected an *Alu* sequence in an intron of the human thymidine kinase gene (HSTKRA) with 81·3% homology and a chimpanzee *Alu* repeat from the cluster 5′ of the alpha 2 globin gene (PTRE 123) with 73·2% homology. Interestingly, the sequenced stretch shows high homology (78·8%) to a human U2 small nuclear RNA pseudogene (HSUG2P). The inverse orientation (to that expected by the orientation of the 517 primer) is especially surprising given the small size of the alphoid-*Alu* fragment in which it was sequenced. This suggests fragmentation/reorganization of sequences at this junction between 2 different classes of repeats, as has been found at amplification joints (Hyrien, *et al.* 1987).

DISCUSSION

Our work on PCR with alphoid repeat-based primers in combination with *Alu* or LINEs

Table 1. *Sequence comparisons of partial sequences from PCR with Y_1 and 517 on the Y chromosome (3E7)*

```
517Y1BAND2          1  TTACTCCCTGTAANCACAGCACTTTGGGAAGCCAAGGTGGGTGGNTCACCTGACGG
ALUCONCENS         16  C  -A       T                G  G                 -
517YBNDIA              ATTCTCCTGTTTCAGCNTCCCTA  TAG  T  AG  C  TAGGNACTACAGGCNTNCGCCACTACGCCTAGCTAA
ALUCONCENS              |  -  -AC A  AAA  A AA    A  T          -C  GCG GGT    GCG   TG  AT  C     |
ALUCONCENS-REV          |      CC     C    - - G                -   G  T      GCG     C       CG    |
HISTKRA                 |       C     C   -G -                  -   G  T      A G     C       C     |
PTRE123                 |      CCC    C    G G    C  G   TAGCT  G              GCC C           G     |
HSUG2P                  |      CA     C    -  -                 -   G          ACGT    C       CG    |
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Gaps in the PCR sequence are placed to allow inserted base pairs in the compared sequences to be shown. Vertical lines indicate ends of the compared sequences; dashes are deletions.

repeat primers expands the approach of utilizing human repetitive sequences to identify and characterize human chromosomal material in somatic cell hybrids. Although we were able to detect distinguishable DNA fragments on total human DNA using chromosome-specific alphoid primers (in combination with other repeat primers), these fragments were not the same as those found when rodent hybrids containing the particular chromosome were used as templates. Thus, the practicality of this approach for identifying the presence of particular chromosomes in total human DNA is moot. Others are using chromosome-specific alphoid primers to enhance chromosome detection on spread chromosomes instead (Koch *et al.* 1989). Both approaches confirm the usefulness of chromosome-specific alphoid repeats for molecular genetics. In somatic cell hybrids, however, the combination of interspersed repetitive element primers and alphoid repeat primers generates novel DNA fragments in the PCR reaction, possibly paving the way for identification of pericentromeric sequences.

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