TECHNICAL NOTE

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Identification of Human DNA in Complex Biological Samples Using the Alu Polymerase Chain Reaction

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ABSTRACT: Alu-Polymerase chain reaction (PCR) was used to amplify human DNA from complex mixed sources of DNA. Amplification of human DNA sequences by Alu-PCR could be accomplished in samples containing low concentrations of template in the presence of excess heterologous DNA sequences. Thus, sensitivity and specificity are maintained in complex DNA mixtures allowing positive identification of the presence of human DNA sequences by this technique.

KEYWORDS: forensic science, Alu polymerase chain reaction (Alu-PCR), human identification, DNA

The polymerase chain reaction (PCR) has proven to be an extremely powerful technique in the molecular analysis of numerous biological processes. DNA analysis of biological evidence in forensic science laboratories has also been augmented by PCR technology (such as HLA-DQα and repeat sequence polymorphism typing, X and Y sequence amplifications, etc.) [*1–4*]. We suggest the use of an Alu-PCR protocol for the identification of human DNA from unknown or mixed DNA sources.

Alu repetitive sequences are the most numerous short interspersed repeats in the human genome, accounting for 3 to 6% of the total genome [*5,6*]. These sequences consist of directly repeating 300 base pair monomer units, with an average distance between copies of approximately 4 kilobases [*5,6*]. Alu repetitive sequences are found in human DNA and some other primate DNA sources [*5,6*], but not in rodents or bacteria. Repetitive sequences have been found in various other species, but these repetitive elements are apparently unrelated to Alu [*6*].

The Alu-polymerase chain reaction (Alu-PCR) was first described by Nelson et al. [*7*]

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who preferentially amplified human DNA from rodent cells that carried individual human chromosomes. Brooks-Wilson et al. [8] developed an Alu-PCR system that used a single Alu primer directed to the extreme 3'-end of a consensus Alu repetitive sequence. With the single primer Alu-PCR methodology, DNA sequences are amplified only if they are between Alu repeats that are in opposite orientation and sufficiently close together (<3 kb apart) [9]. There are numerous sites in the human genome that possess these necessary characteristics for amplification by this technology [8]. Nonetheless, only a small subset of the total human genomic DNA is amplified by the single primer technique producing a complex pattern of individual DNA bands that can be discerned by agarose gel electrophoresis [7,9]. Alu-PCR has been used to detect extrachromosomal DNA in tumor cells [10] and most recently we have used this technique to amplify putative nuclear matrix attachment regions in human fibroblasts [9]. We now propose that a DNA specimen can be identified as originating from a human source even if present in small quantities and contaminated with non-primate DNA by using PCR primed with an Alu repetitive element.

Materials and Methods

Cell Lines

Human fibroblasts derived from human foreskin and transformed with SV40 [11] were maintained in a humidified atmosphere of 5% CO_2-95% air at 37°C. Rat liver epithelial cells, which carry individual human chromosomes, provided a source for complex DNA mixtures and were maintained as described [12]. Individual human chromosomes (human chromosome 2, 11, or 13, which carry the neomycin resistance gene) were introduced into a tumorigenic variant (GN6TF) of WB-F344 rat liver epithelial cells via microcell mediated chromosome transfer [13]. Neomycin-resistant colonies were isolated using cloning rings and established as pure clones.

DNA Isolation

DNA was isolated from cells maintained in tissue culture using standard laboratory protocols. SV40 transformed human fibroblasts served as a source of pure human DNA and rat liver epithelial cells that carry individual human chromosomes provided DNA in complex mixtures. Briefly, cell pellets were resuspended in lysis buffer containing 200 μg/mL proteinase K and incubated for at least 2 h at 37°C. All samples were extracted with equal volumes phenol:chloroform (1:1) followed by chloroform:isoamyl alcohol (24:1). The recovered aqueous phases were treated with RNase (100 μg/mL) for 1 h and extracted as above. DNA was precipitated in 0.1 volumes of 3M sodium acetate and 2.5 volumes of ethanol at −20°C.

Bacterial Strains and Isolation of Bacterial DNA

Bacterial DNA was prepared from Escherichia coli (Strain DH5α) or from Enterobacter cloacae that had been propogated in M9 minimal essential media [14]. DNA was prepared from these bacterial strains as described [14].

Alu-PCR Amplification

Alu-PCR was carried out according to the protocol of Brooks-Wilson et al. [8]. Briefly, various concentrations of isolated human genomic DNA (10 pg to 100 ng) was used as a template source in the PCR reaction. In some cases, human template DNA was mixed

with an excess of bacterial DNA or was contained within a complex mixture containing rodent DNA. The DNA (up to 200 ng total DNA per reaction) was incubated in a total reaction volume of 100 μl containing Alu primer (0.5 μM) (GCGAGACTCCATCT-CAAA), 0.25 μM of each dNTP, 25 mM Tris-HCl (pH 8.3), 25 mM KCl, 1.5 mM $MgCl_2$, and 2.5 units Thermus aquaticus polymerase (Perkin-Elmer/Cetus) [8]. DNA samples were denatured at 94°C for 8 min prior to PCR. PCR amplification was accomplished using 35 cycles consisting of a 2 min denaturation step (94°C), 2 min primer annealing step (56°C), and a 3 min extension step (72°C). The final cycle included a 10 min extension step. The samples were then extracted with chloroform/isoamyl alcohol (24:1) and precipitated in 2 volumes of isopropanol at −20°C in the presence of 300 mM sodium acetate. The DNA pellets were dissolved in 10 mM Tris/1 mM EDTA (pH 7.6) buffer and analyzed by gel electrophoresis on 2% agarose gels containing 40 mM Tris-acetate/1 mM EDTA (pH 7.6).

Results and Discussion

Alu-PCR can be used as a rapid and unique method for identification of human DNA within complex biological samples. We demonstrate that Alu-PCR amplification of human genomic DNA isolated from tissue culture cells results in distinct bands of DNA when electrophoretically separated in agarose gels (Fig. 1). The amplified fragments range in size from 0.5 to 3 kb. The observation that discrete bands result from Alu-PCR of human DNA emphasizes the importance of location of amplifiable DNA sequences between two Alu repeats that are in opposite orientation and within several kb of one another. We found that varying the concentration of purified human DNA from 50 ng to as little as 1 ng for use as template resulted in easily discernible banding patterns by ethidium bromide staining of agarose electrophoresis gels (Fig. 1). We have further established that high sensitivity can be accomplished when only 10 pg of pure human genomic DNA is used as template for Alu-PCR. However, the agarose gel pattern of Alu-PCR amplified DNA from small quantities of human DNA template were observed to contain fewer DNA bands than the patterns obtained when greater amounts of template were included

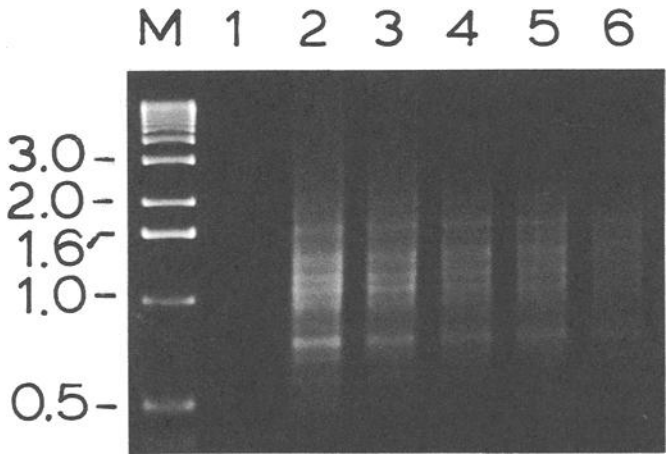

FIG. 1—*Alu-PCR of genomic DNA isolated from SV-40 transformed human fibroblasts. Template DNA, 50 ng (Lane 2), 10 ng (Lane 3), 5 ng (Lane 4), 3 ng (Lane 5), or 1 ng (Lane 6), was amplified using a single primer to the human Alu repetitive sequence. Lane 1, control containing no template DNA. Molecular size markers (M) are shown with respective sizes (kb) at left.*

in the reaction, possibly because the minor amplification products falls below the detection limit for ethidium bromide.

Alu-PCR is similar to other PCR protocols that use forensically valuable loci in that there need not be concern for the presence of contaminating DNA sequences, such as those from microbial or other animal sources. However, Alu-PCR has great advantage over routine PCR procedures that amplify a specific DNA sequence requiring the presence of two unique primer sequences in the presence of an excess of heterologous DNA sequences which lack these primer target sequences [*16*]. Alu-PCR amplifies random DNA sequences of human origin using multiple primer target sequences in samples containing excess DNA from other sources that lack the Alu repeat. Thus, amplification with this method relies upon the absence of Alu sequences in contaminating DNA sources and is less sensitive to inhibition by autolysis or genomic fragmentation. A computer search of all available sequences in Version 71 of the Genbank (using Eugene Release 3.2, Mol. Biol. Info. Resource, Baylor College of Medicine) failed to identify DNA sequences with homology to the Alu primers used in this study in species other than human and other select primates (Table 1). This suggests that the design of the Alu primer used in this study is sufficiently specific to human and primate species to ensure that heterologous DNA from other sources would not be amplified by this method. However, it is possible that there are sequences not in Genbank, which may have homology to the primers used here.

Alu-PCR amplification of human DNA sequences from complex DNA sources demonstrates the utility of this method for forensic science applications. Specific amplification of human sequences can be accomplished from human chromosome 2, 11 or 13 DNA introduced into rodent epithelial cells (Fig. 2) or from mixtures of human DNA with

TABLE 1—*Alu repetitive sequences from human and nonhuman DNA sources.*[a]

Category Searched	Species	Gene Designation[b]	Accession Number	Number of Sequences[c]
Human				>500
Primate[d]				23
	Chimpanzee	CHPHBBPCH	K02542M18075	
	Gibbon	GIBBGLDETA	M54985	
	Orangatan	ORAEGLOG	X05035	
	African Green Monkey	AGMRSASAT	J0034V00142	
	Baboon	BABAPDE	M29322	
	Gorilla	GORAW	X06123	
Rodent				0
Mammal[e]				0
Vertebrate[f]				0
Invertebrate				0
Bacteria				0
Eukaryotic virus				0
Phage				0
Plant				0

[a]Version 71 of the Genbank was searched using Eugene Release 3.4 (Mol.Biol.Info. Resources, Baylor College of Medicine). The entire 18 nucleotide sequence of the Alu primer (GCGAGACTCCATCTCAAA) was used to search all categories of organisms in the gene bank.

[b]Representative gene sequence matched from the primate DNA sequence data base.

[c]Number of sequences found allowing up to two nucleotide mismatches within the searched sequences.

[d]Excludes human sequences.

[e]Excludes primate and rodent sequences.

[f]Excludes mammalian sequences.

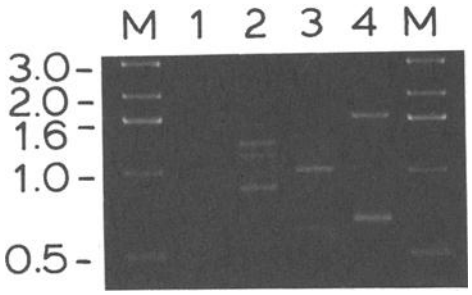

FIG. 2—*Alu-PCR of DNA isolated from rat liver epithelial cells that carry human chromosomes 2, 11 or 13. Template DNA (50 ng) was amplified by PCR using a single primer to the human Alu repetitive sequences. Lane 1, rat genomic DNA only; Lane 2, clone GN6TF-11 neo C1 (human chromosome 11); lane 3, clone GN6TF-13 neo C2 (human chromosome 13); lane 4, clone GN6TF-2 neo C2 (chromosome 2). Molecular size markers are shown with respective sizes (kb) at left.*

bacterial DNA (Fig. 3). DNA from rat liver epithelial cells produces no Alu-PCR amplification products due to the absence of Alu sequences in the rodent DNA [6] (Fig. 2, lane 1). However, Alu-PCR of the same cells that carry human chromosome 2 (Fig. 2, lane 4), 11 (Fig. 2, lane 2) or 13 (Fig. 2, lane 3) produce prominent amplification patterns. These results demonstrate that human sequences are efficiently amplified from small samples of DNA carrying excess mammalian DNA that is not of human origin. In addition, specific Alu-PCR amplification of the human DNA can be accomplished in the presence of bacterial DNA. DNA from SV-40 transformed human fibroblasts was mixed with Escherichia coli (Fig. 3) or Enterobacter cloacae (Data not shown) genomic DNA in various proportions and Alu-PCR was performed. We observed that amplification could be accomplished with as little as 20 pg human DNA template in the presence of 5000-fold excess of bacterial DNA, without amplification of bacterial DNA sequences (Fig. 3). Thus, Alu-PCR facilitates identification of human DNA in complex mixtures of DNA.

Advances in PCR technology have eliminated the requirement for highly purified DNA template. Amplification can be accomplished from whole cell lysates or small samples

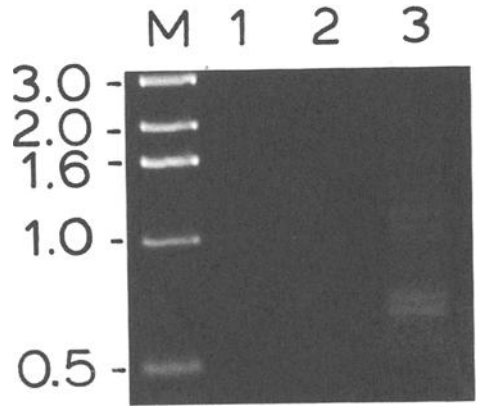

FIG. 3—*Alu-PCR of human genomic DNA from complex mixtures containing bacterial genomic DNA. Lane 1, control reaction containing no human or E. coli template DNA; Lane 2, 100 ng E. coli genomic DNA only; Lane 3, mixture of human and E. coli genomic DNA (20 pg human DNA, 100 ng E. coli DNA). Molecular size markers are shown with respective sizes (kb) at left.*

prepared from blood or other body fluids [*16*]. Due to the nature of Alu-PCR amplification and the multiple amplification sites, there is less concern for the possibility of DNA degradation in somewhat autolyzed tissues. Thus, it is likely that this technique would require less time and materials than other DNA analysis protocols used in the forensic laboratory. Therefore, we suggest the use of this Alu-PCR technique as an aid in the identification of human DNA from complex or unknown samples.

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