Gabriel E. Novick[1]
Corina C. Novick[1]
Juan Yunis[2]
Emilio Yunis[3]
Kianfa Martinez[1]
George G. Duncan[4]
Gary M. Troup[5]
Prescott L. Deininger[6]
Mark Stoneking[7]
Mark A. Batzer[8]
Rene J. Herrera[1]

[1]Department of Biological Sciences, Florida International University
[2]Dana-Farber Cancer Institute, Department of Pathology, Harvard Medical School
[3]Instituto de Genetica, Universidad Nacional de Colombia
[4]Broward County Sheriff's Office, Crime Lab
[5]Department of Pathology, School of Medicine, University of New Mexico, New Mexico
[6]Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center
[7]Department of Anthropology, Pennsylvania State University
[8]Human Genome Center, L-452, Biology and Biotechnology Research Program Lawrence Livermore National Laboratory

Polymorphic human specific *Alu* insertions as markers for human identification

Alu sequences represent the largest family of short interspersed repetitive elements (SINEs) in humans with 500 000 copies per genome. Recently, one *Alu* subfamily was found to be human specific (HS). We originally described the use of polymorphic HS *Alu* insertions as a tool in population studies and recently as tools in DNA fingerprinting and forensic analysis. In this report, we will use this simple polymerase chain reaction (PCR) base technique for the detection of HS *Alu* insertion polymorphisms. We will test the resolving power of this DNA profiling approach in both population genetics and paternity assessment. At the population level, we will describe the genotypic distribution of five polymorphic *Alu* insertions among 3 populations from the American continent, one of African origin, the other two Amerindians. Insight into their relationships will be provided. At the family level, we will examine one European American family of seven individuals and the same pedigree will also be characterized by way of the two systems currently and widely used to ascertain paternity: PCR-sequence specific oligonucleotide probe hybridization (PCR-SSO) and PCR-restriction fragment length polymorphism (PCR-RFLP) of human leucocyte antigen (HLA) class II molecules, and a standard RFLP protocol used in forensic casework and paternity studies. The importance and strengths of the method as well as its perspectives for future use in filiation studies will be evaluated.

1 Introduction

Human genetic diversity is the result of the differential accumulation of mutations throughout evolution in individuals or populations. The identification of those distinctive characteristics in the DNA represents the basis for human identification and population genetics. Some of the early attempts to classify individuals and populations based upon variation at the molecular level were the studies of blood types in humans. Restriction fragment length polymorphisms or RFLPs [1], hypervariable minisatellite regions [2] and human leucocyte antigen (HLA) typing [3], constitute the three most widely used DNA based identification strategies today. There are several major levels of identification that DNA typing approaches could be applied to. Some of the best known applications include individual differentiation, paternity assessment and population or geographical relationships which in turn may include the study of population structure, migration and evolution. Not all of the above mentioned techniques, however, can be used to answer the same type of questions and the degree of variability observed by the different procedures will limit their optimum range of usage. For example, ABO typing would not be the method of choice to discriminate between individuals because many people share the same phenotype/genotype. On the other hand, although VNTRs, may offer the optimal resolution for forensic casework or paternity testing, extensive hypervariability at such loci limits the magnitude of interpopulational variation.

The *Alu* family of repetitive elements was originally defined as a fraction of renatured repetitive DNA that was distinctively cleaved with the restriction enzyme *Alu*I [4]. The family is present in an excess of 500 000 copies per haploid genome [5] and constitutes approximately 5% of the total mass of the genome [6]. The basic structure of an *Alu* element (Fig. 1) shows a dimeric organization [7] in which the right monomer is 31 nucleotides longer than the left one. The left half of the element contains the typical RNA polymerase III split promoter (boxes A and B) [8]. They show a short A rich linker between the two dimers and up to 100 bp polymorphic 3′ oligo-dA rich tail characteristic of all SINEs [9]. The *Alu* repeat is flanked by direct repeats derived by duplication of target sequences at the site of integration.

Correspondence: Rene J. Herrera, Ph. D. Florida International University, University Park Campus, Miami, FL 33199, USA (Tel: +305-348-1258; Fax: +305-348-1259)

Nonstandard abbreviations: **HLA,** human leukocyte antigen; **HS,** human specific; **SINE,** short interspersed elements; **SSO,** sequence-specific oligonucleotide probe hybridization

Keywords: *Alu* repeats / Population genetics / Paternity testing

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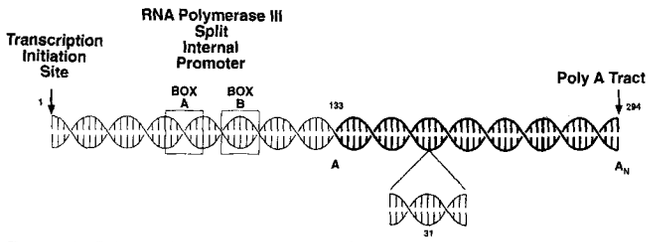

Figure 1. Schematic representation of the structure of a generic *Alu* repeat. Represented are the two monomers, the 31 bp insertion in the left monomer, the internal and 3′ adenine rich track, the flanking direct repeats (arrows) and the split internal promoter (boxes A and B) of the polymerase III. Light and heavy lines represent the two halfs of the dimeric structure.

Alu elements are derived from the 7SL RNA gene and they share about 90% sequence homology with 150 bp in the middle that is not found in the *Alu* familiy [10]. These elements mobilize by retroposition through an RNA polymerase III intermediate [11] and their chromosomal distribution show certain preferences for R bands or AT rich areas [12]. *Alu* elements first appeared in primate genomes about 65 million years ago [13] and have since undergone amplification from a few master genes [14] to their repetitive status today at a rate of approximately 8×10^{-3} *Alu* elements per year [15] with about 5×10^{-7} nucleotide substitutions per year [15]. *Alu* elements are considered members of the same family if they have 20–30% or less divergence from the consensus, while they are grouped in the same subfamily if they have only 4% or less divergence between them [7]. One of those subfamilies was found to be human specific (HS) [16], and actively retrotransposing into new genomic locations [17].

Recently, we described the use of polymorphic *Alu* insertions in human DNA fingerprinting [18]. In this report, we demonstrate the use of a simple PCR base technique for the detection of HS *Alu* insertion polymorphisms. We will test the usefulness of this fingerprinting approach in two of the three major levels of identification described above: population genetics and paternity assessment. At the population level, we will describe the genotypic distribution of five polymorphic *Alu* insertions (loci) in 3 populations from the American continent. Some information about their relationships and migrational patterns will be presented. At the family level, we will examine one European American family of seven individuals and the same pedigree will also be characterized by way of the two of the most widely used systems to ascertain paternity: PCR-SSO (sequence specific oligonucleotide probe hybridization) and PCR-RFLP of HLA class II molecules, and a standard RFLP protocol used in forensic casework and paternity studies. The importance and strengths of the method as well as its perspectives for future use in filiation studies will be evaluated.

2 Materials and methods

2.1 Source of DNA

DNA from peripheral blood mononuclear cells was isolated as previously described [19]. The geographic localization of the populations studied is shown in Fig. 2.

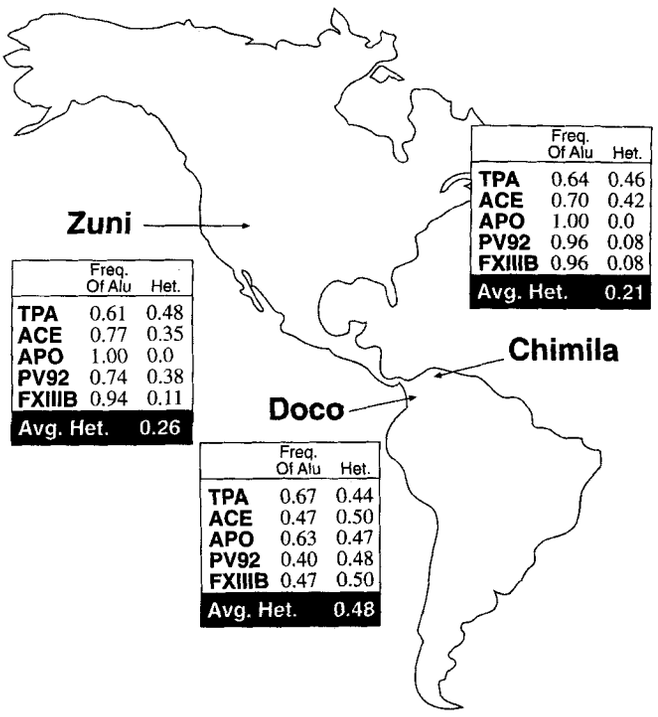

Figure 2. Map of the American continent showing the geographical distribution of the 3 populations surveyed, their *Alu* insertion frequencies and levels of heterozygosities. The groups studied were: Doco (an African population from northwestern Colombia), Chimila (an Amerindian population from northeastern Colombia) and Zuni (an Amerindian population from southwestern United States).

They are: Doco from northwest Colombia, Chimila from northeast Colombia and Zuni from southeast USA. A European American family was used for the comparative paternity testing analysis.

2.2 RFLPs polymorphism procedure

DNA samples were analyzed using the FBI standard RFLP protocol [20, 21]. The sizing standard was a 30 band, 0.6 to 23 kbp, λ DNA ladder. The gels were run for 16 h at 30 V in 1 × TAE (Tris-acetate-EDTA) buffer. The gels were then Southern blotted onto positively charged nylon membranes, using 0.4 M NaOH as transfer solution and probed with the following probes: YNH24 (locus D2S44), TBQ7 (locus D10S28); MS1 (locus D1S7), PH30 (locus D4S139) and pLH1 (locus D5S110).

2.3 HLA Polymorphism procedure

DNA samples were amplified by PCR for DRB generic, DQA1 and DQB1 loci in 100 μL reaction mixtures containing 50 pmoles of each primer, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 200 μM of each deoxynucleotide, 2.5 units of *Taq* polymerase and 1.5–2 mM $MgCl_2$. The primers and conditions used in this study as well as dot blots, prehybridization and hybridization procedures were as previously described [22, 23]. The DRB, DQA1 and DQB1 alleles were determined using the locus-specific PCR amplified products by PCR-SSO as described before [22, 23].

2.4 HS *Alu* PCR polymorphism procedure

2.4.1 Oligonucleotide primers

Five different *Alu* specific sets of primers were used: ACE, TPA25, PV92, APO and FXIIIB. Each pair is directed to the 3′ and 5′ single copy flanking sequences of one of the five different *Alu* insertions. Primer location and sequences have been described elsewhere [24]. In each case, the HS *Alu* elements reported here have in fact been shown to be unique to the human lineage and absent from orthologous positions within the great apes and other primate genomes [25].

2.4.2 PCR and electrophoresis conditions

PCR amplification was carried out in a final volume of 100 μL as previously described [25] and 10 μL of PCR products were electrophoresed in 3% TAE agarose gels with 0.5 μg of Hae III digested ΦX 174 DNA as molecular weight marker. DNA fragments were visualized after staining in ethidium bromide.

2.4.3 Data analysis

Allelic frequencies were calculated using the gene counting method and tested for Hardy-Weinberg equilibrium using the chi-square test for goodness of fit. Unbiased estimates of average heterozygosity and the associated standard error were calculated as previously described [26].

3 Results

The principle of the assay is summarized in Fig. 3. DNA banding patterns were scored according to the following criteria. Homozygous individuals for the insertion exhibit one band approximately 400 bp (TPA25, APO and PV92), 490 bp (ACE) or 700 bp (FXIIIB) long. In other words, the *Alu* insertion is present at the locus in both homologous chromosomes. Homozygous individuals for the lack of insertion allele are represented by one band approximately 110 bp (TPA25, APO and PV92), 190 bp (ACE) or 500 bp (FXIIIB) long. In these individuals the element is not present in either of the two homologous chromosomes. Heterozygous individuals exhibit both bands, one of each of the two fragment lengths. Five HS *Alu* polymorphic loci were analyzed for both the population and paternity studies.

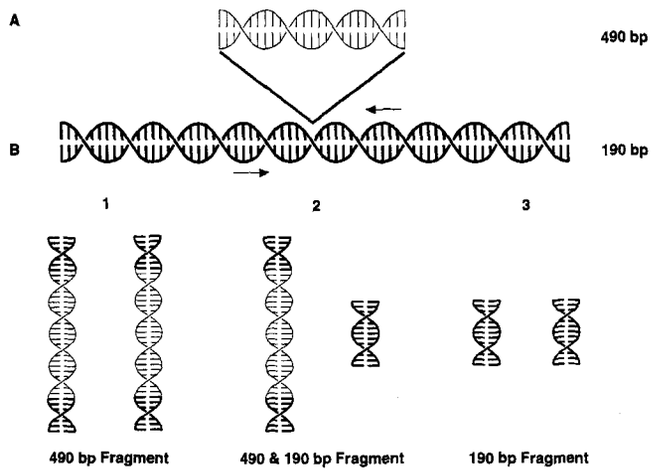

Figure 3. Principle of the PCR based *Alu* polymorphism assay and resulting bands according to genotype. Part "A" at the top illustrates an *Alu* insertion and the size of the amplification product of an insertion allele (490 bp). Part "B" represents the amplification and size of a lack of insertion allele (190 bp). Bottom panel represents the sizes of the PCR products for the three possible genotypes. Light lines represent *Alu* sequences while heavy lines illustrate flanking genomic DNA.

3.1 Population analysis

A total of 81 individuals from 3 different populations were tested. The allele frequencies and the average heterozygosity for each population are shown in Fig. 2. Except for APO which was fixed for the presence of the *Alu* insertion in the Chimila and Zuni, all other loci were polymorphic in all 3 populations studied. The allelic frequencies of the *Alu* insertion for all loci and populations studied are shown in Figure 2. The heterozygosity for each polymorphic locus in each population ranged from 0.08 for PV 92 and FXIIIB in the Chimila to 0.5 for ACE and FXIIIB in the Doco group (Fig. 2). The average heterozygosity for all three groups and all loci studied ranged from 0.21 in the Chimila to 0.48 in the Doco (Fig. 2).

3.2 Pedigree analysis

To establish the probability of paternity for the family represented in the pedigree, DNA fingerprints of the mother, children and father were compared (Fig. 4). The paternity index is defined as the ratio of the chance of paternity for a possible biological parent to the chance of paternity for a randomly selected individual of the appropriate population. The probability of paternity is the expression of the paternity index as a percentage [27]. For the RFLP analysis, paternal and maternal bands were compared with the profiles of the children. All of the nonmaternal bands in the child's DNA fingerprint had to be present in the father for paternity to be assigned. The lower portion of Fig. 4 represents an autoradiography with the banding profiles of the individuals in the pedigree using the RFLP/VNTR probe D5S110. Five DNA RFLP/VNTR probes were used to establish paternity. In this family, paternity was confirmed for each child at a probability of paternity greater than 99.99% using methods suggested by the American Association of Blood Banks [27].

The HLA Polymorphism study of this family only comprised DRB generic and DQA1 allele typing. The segregation of haplotypes are shown in Fig. 5. The father (WA11) carried haplotypes a and b, while the mother (WA16) carried haplotypes c and d. The expected linkage disequilibrium was detected in all 4 haplotypes. DRB1*03 and DRB1*11 are in linkage disequilibrium in almost 100% of the cases with DQA1*0501. Similarly, in most cases in the general population DRB1*04 segregates together with DQA1*03 while DRB1*01 is in linkage disequilibrium with DQA1*0101. Probability of paternity was calculated as described above and the values obtained were 82.25% (DRB1*01, DQA1*0101)

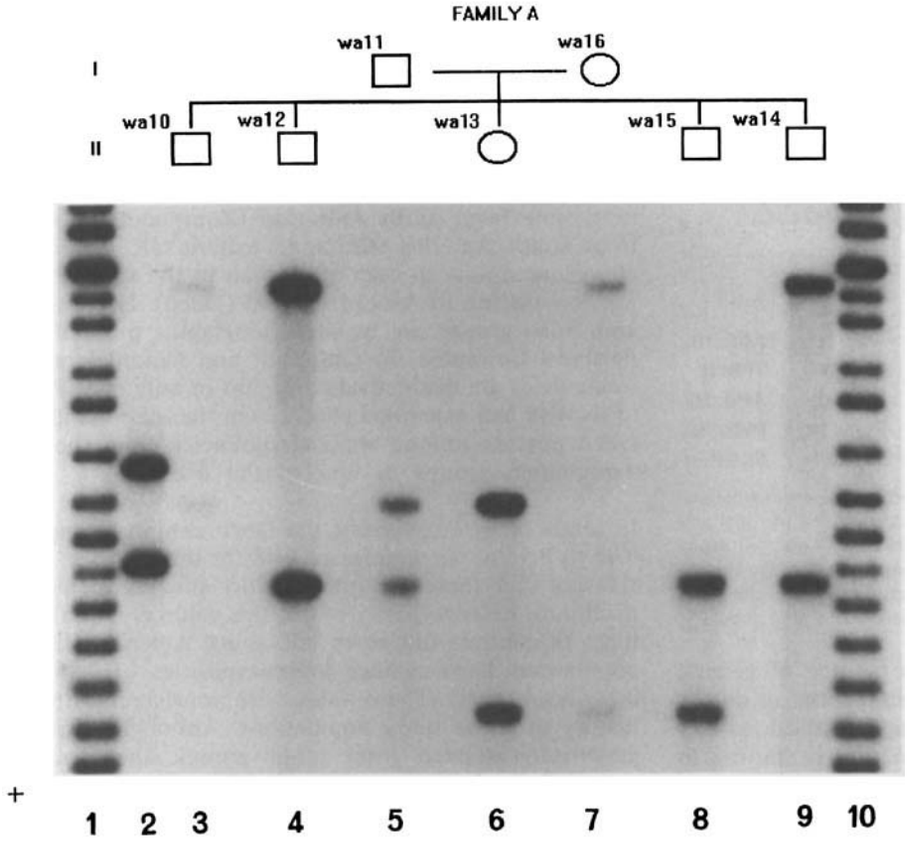

Figure 4. Pedigree showing the RFLP/VNTR banding profile from family members. Roman and Arabic numerals in the pedigree indicate generations and individuals, respectively. Numbers under the autoradiograph show the samples loaded on the gel. Wells 1 and 10 contain molecular weight/size markers (see Section 2). Well 2 demonstrates the RFLP/VNTR banding pattern for the human cell line K562 as a standard. Wells 3 to 9 represent the DNA profiles of the family members. Notice that each banding pattern is directly below the corresponding square or circle symbol for each family member in the pedigree. Each individual exhibits two bands one inherited from the father and the other from the mother. The different band locations reflect different migration rates which, in turn, is the result of different number of tandem repeats. Individuals WA11 and WA16 represent the father and mother in this pedigree. Wells 5 and 7 exhibit the banding pattern of the two heterozygous parents with two bands as opposed to single bands found in homozygotes. The offspring in wells 3, 4, 6, 8, and 9 are also heterozygotes since they inherited different size fragments from the mother and the other from the father. Notice that mother and father possess unique bands (bands are not shared).

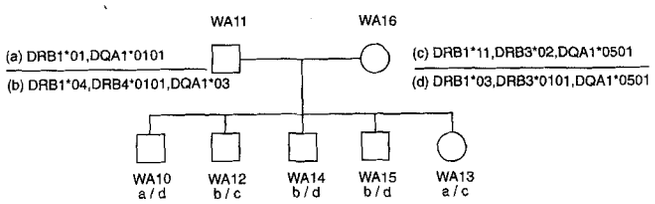

Figure 5. Diagram showing the pedigree of the family studied demonstrating the segregation of HLA DRB and DQA1 haplotypes.

and 85% (DRB1*04, DQA1*03) depending on the haplotype frequency in the general population.

The HS *Alu* PCR polymorphism analysis was based on the allelic distribution of the five loci studied (TPA25, ACE, APO, PV92 and FXIIIB). The banding profiles for the ACE locus for the seven individuals in this family after agarose gel electrophoresis are shown in Fig. 6. The different banding patterns that result from this diallelic locus are represented. Figure 7 summarizes the genotypes for the five polymorphic loci. The likelihood of paternity or maternity with respect to the five offspring in the pedigree was calculated as previously described. For the parents of this pedigree, the probability of paternity and maternity was 89% and 96%, respectively.

4 Discussion

Alu retroposition constitutes an ongoing valuable source of human specific stable genetic variability. *Alu* elements, rather than useless sequences as they originally were

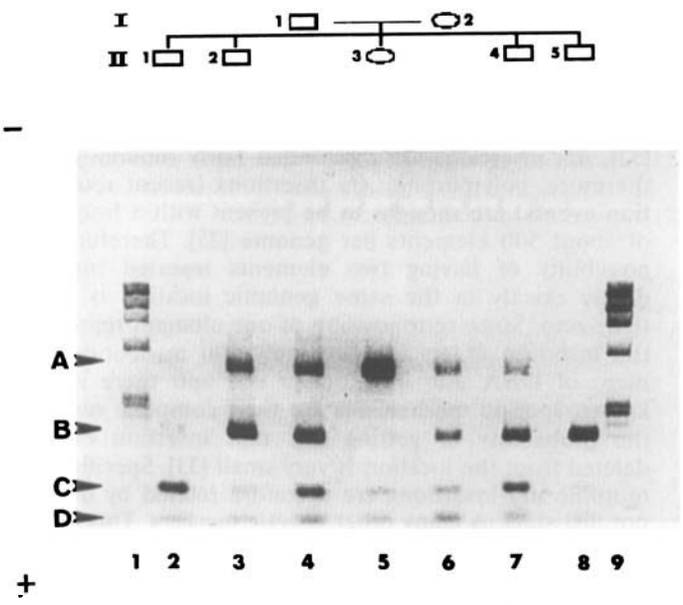

Figure 6. Agarose gel electrophoresis of the *Alu* PCR amplification products for the ACE locus from members of the family analyzed. On the gel: lanes (1) and (9) marker, *Hae*III digested ΦX174; (2) individual II1; (3) individual II2; (4) individual I1; (5) individual II3; (6) individual I2; (7) individual II4; (8) individual II5. The insertion band is seen at the level of arrow "A" (490 bp) and the lack of insertion band at the level of arrow "B" (190 bp). The fastest (labeled "D") and the second fastest (labeled "C") moving bands are primer and primer-dimer, respectively. The scores are as follow: homozygous for the lack of insertion, II5; homozygous for the insertions, II3 and heterozygous, I1, I2 II1, II2, II4.

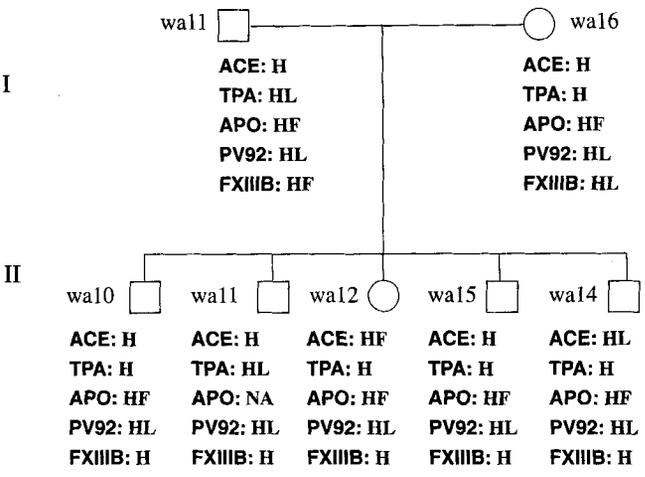

Figure 7. Pedigree of the family studied showing the scores obtained after HS *Alu* PCR amplification analysis for each of the five loci analyzed. HF: Homozygous for the insertion (a HS *Alu* is inserted in the same locus in both members of the chromosome pair): H: Heterozygous; HL: Homozygous for the lack of insertion (none of the members of the chromosome pair have an insertion in that locus).

thought to be, represent a dynamic source of genetic variation in most cases either inconsequential or deleterious with dramatic corresponding medical consequences to the individuals. This variation is starting to be also appreciated as raw material for several important evolutionary processes [28]. A search for the presence of *Alu* elements within over 100 000 sequences showed 17 *Alu* insertions within exons of 15 different cDNAs [29]. Meningiomas [30], hemophilia B in hemizygous males [31] and neurofibromatosis type 1 [17], among others, are all diseases associated with the presence of one or several *Alu* elements implicated in different molecular rearrangements that include, recombination, intron sliding, alternate splicing and duplication. Although there may be some insertional preferences at A + T rich regions [32], *Alu* insertions are distributed fairly randomly. Furthermore, polymorphic *Alu* insertions (recent retroposition events) are thought to be present with a frequency of about 500 elements per genome [25]. Therefore, the possibility of having two elements inserted independently exactly in the same genomic location is essentially zero. Since retroposition of one element represents the insertion of an approximately 300 nucleotide fragment of DNA into a particular site and there are no known specific mechanisms for their complete removal, the probability of getting just that insertion entirely deleted from the location is very small [33]. Specific polymorphic *Alu* insertions are therefore related by descent not just state as many other genetic markers. This makes *Alu* elements excellent markers for both population and paternity studies. Many *Alu* insertions, in fact, represent ancient events or molecular fossils which remain for the most part intact after millions of years. The oldest *Alu* insertions are about 65 million years old, while the oldest HS *Alu* insertions are approximately 2.8 million years old. This type of genetic marker allows for more precise phylogenetic reconstructions and it may prove to be a valuable tool in the field of molecular archaeology. In a recent report [34], we assessed the validity of the *Alu* insertion polymorphism data to study population relationships by calculating G_{st} values (measure of the relative magnitude of genetic differentiation among groups) in 664 individuals from 16 populations. Significant differences among populations were found. In the present study, we describe the allele frequencies of five polymorphic *Alu* insertion loci in three populations from the American continent, two Amerindian and one of African origin. As expected, the American Indian populations, one from north American (Zuni) and the other from south America (Chimila), exhibit allelic frequencies more similar to each other than to the south American population of African descent (Doco). The Chimila and Doco groups are in close geographic proximity in northern Colombia. In fact Zuni and Chimila's allelic frequencies are distinctively different in only one (PV92) of the five loci examined (Fig. 2). On the other hand, the Doco possess unique allelic frequencies from the two Amerindian groups in four of the five loci.

In terms of heterozygosity, the Doco exhibit a range of 0.44 to 0.5 and an average of 0.48 for the five loci. Considering that these are biallelic loci and hence have a maximum heterozygosity of 0.5, this value is remarkably high. In contrast the north and south American Indian populations have average heterozygosities of 0.26 and 0.21, respectively. These values are consistent with the history of these three populations. Although currently genetically isolated from other groups, the Doco is expected to have experienced admixture from Caucasian and neighboring Amerindian populations since their introduction into a mining area of northwestern Colombia four centuries ago most likely from western Africa. The Zuni, the Chimilas and their ancestors that migrated to the new world, on the other hand, probably were subjected to bottleneck effects as they settled in their current locations. Subsequently, these Amerindian populations have been procreating to a great extent in isolation and to some degree under inbreeding conditions.

Noteworthy is that the frequencies of *Alu* insertions in the Doco population (TPA, 0.67; ACE, 0.47; APO, 0.63; PV 92, 0.40; FXIIIB, 0.47) are distinctly different from the frequencies in Nigerians (TPA, 0.41; ACE, 0.27; APO, 0.5; PV 92, 0.09; FXIIIB, data to appear elsewhere) [34], a west African population related to the original African slaves brought to America. The Nigerians were found to be the closest to a hypothetical ancestral human population with an *Alu* insertion frequencies set at zero (the ancestral state) for four of the five polymorphic insertions reported here [34]. Since the frequencies of these *Alu* insertions in Doco are intermediate between the Nigerian, on one hand, and the Amerindian (Chimila: TPA, 0.64; ACE, 0.70; APO, 1.00; PV 92, 0.96; FXIIIB, 0.96 / Zuni: TPA, 0.61; ACE, 0.77; APO, 1.00; PV 92, 0.74; FXIIIB, 0.94) and European American (TPA, 0.56; ACE, 0.51; APO, 0.94; PV 92, 0.18; FXIIIB, data to appear elsewhere) [34] populations on the other, this data is consistent with historical records and gene flow from American Indian and/or Caucasian populations into the Doco population subsequent to their introduction in present day Colombia.

In the filiation study, we compare the discriminative power of HS *Alu* insertion polymorphisms with respect

to HLA analysis and RFLPs/VNTR profiling. With only five loci, the *Alu* insertion system yielded a percentage probability of paternity and maternity of 89% and 96%, respectively [35]. It is calculated that there are between 500 and 2000 HS *Alu* repeats [36]. Considering that many of these loci may be polymorphic *Alu* insertions, the potential of this technology in medical diagnostics and epidemiology, forensic sciences, molecular archaeology and human identification in general is remarkable. The PCR based test for HS *Alu* polymorphisms represents a simple, fast, non-isotopic and easily reproducible system for DNA profiling analysis. At both the levels of population studies and paternity testing, this approach has the potential to not only efficiently complement results obtained with other systems but also by itself, due to its uniquely beneficial characteristics, be a highly accurate tool in human identification.

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5 References

[1] Kan, Y. W., Dozy, A., *Proc. Natl. Acad. Sci. USA* 1978, *75*, 5631–5635.
[2] Jeffreys, A. J., Wilson, V., Thein, S. L., *Nature* 1985, *314*, 67–73.
[3] Dausset, J., *Science* 1981, *213*, 1469–1474.
[4] Houck, C. M., Rinehart, F. P., Schmid, C. W., *J. Mol. Bio.* 1979, *132*, 289–306.
[5] Rinehart, F. P., Ritch, T. G., Deininger, P. L., Schmid, C. W., *Biochemistry.* 1981, *20*, 3003–3010.
[6] Krayev, A. S., Markusheva, T. V., Kramerov, D. A., Ryskov, A. P., Skryabin, K. G., *Nucleic Acids Res.* 1982, *10*, 7461–7475.
[7] Deininger, P. L., Jolly, D. J., Rubin, C. M., Friedmann, T., Schmid, C. W., *J. Mol. Bio.* 1981, *151*, 17–33.
[8] Paolella, G., Lucero, M. A., Murphy, M. H., Baralle, F. E., *EMBO J.* 1983, *2*, 691–696.
[9] Economou, E. P., Bergen, A. W., Warren, A. L., Antonarakis, S. E., *Proc. Natl. Acad. Sci. USA* 1990, *87*, 2951–2954.
[10] Ullu, E., Tschudi, D., *Nature* 1984, *318*, 171–172.
[11] Rogers, J., *Int. Rev. Cytol.* 1985, *93*, 187–279.
[12] Korenberg, J. R., Rykowski, M. C., *Cell* 1988, *53*, 391–400.
[13] Deininger, P. L., Daniels, G. R., *Trends. Genet.* 1986, *2*, 76–80.
[14] Deininger, P. L., Batzer, M. A., Hutchinson III, C., Edgell, M. H., *Trends. Genet.* 1992, *8*, 307–312.
[15] Zuckerkandl, E., Latter, G., Jurka, J., *J. Mol. Evol.* 1989, *29*, 504–512.
[16] Batzer, M. A., Deininger, P. L., *Genomics* 1991, *9*, 481–487.
[17] Wallace, M. R., Andersen, L. B., Saulino, A. M., Gregory, P. E., Glover, T. W., Collins, F. S., *Nature* 1991, *353*, 864–866.
[18] Novick, G. E., Gonzalez, T., Garrison, J., Novick, C. C., Batzer, M. A., Deininger, P. L., Herrera, R. J., in: Pena, S. D. J., Chakraborty, R., Epplen, J. T., Jeffreys, A. J. (Eds.), *DNA Fingerprinting: State of the Science*, Birkhäuser Verlag, Basel 1993, p. 283.
[19] Ausabel, F. M., Brent, R., Kingston, U. E., Moore, D. D., Seldman, J. G., Smith, J. A., Struhl, K., (Eds.). *Current Protocols in Molecular Biology*, John Wiley and Sons, New York 1987.
[20] Adams, D. E., *Crime Lab Digest.* 1988, *15*, 106–108.
[21] Budowle, B., Baechtel, S. F., *Appl. Theor. Electrophoresis* 1990, *1*, 181–187.
[22] Salazar, M., Yunis, J. J., Delgado, M. B., Bing, D., Yunis, E. J., *Tissue Antigens* 1992, *40*, 116–123.
[23] Yunis, J. J., Salazar, M., Delgado, M. B., Alper, C. A., Bing, H. D., Yunis, E. J., *Tissue Antigens* 1993, *41*, 37–41.
[24] Batzer, M. A., Arcot, S. S., Phinney, J. W., Alegria-Hartman, M., Kass, D. H., Milligan, S. M., Kimpton, C., Gill, P., Hochmeister, M., Ioannou, P. A., Herrera, R. J., Boudreau, D. A. Scheer, D., Keats, B. J. B., Deininger, P. L., Stoneking, M., *J. Mol. Evol.* 1995, in press.
[25] Batzer, M. A., Gudi, V. A., Mena, J. C., Foltz, D. W., Herrera, R. J., Deininger, P. L., *Nucleic Acids Res.* 1991, *19*, 3619–3623.
[26] Nei, M., *Molecular Evolutionary Genetics*, Columbia University Press, New York 1987.
[27] Gjertson, D. W., Mickey, M. R., Hopfield, J., Takenouchi, T., Terasaki, P. I., *Am. J. Hum. Genet.* 1988, *43*, 860–869.
[28] von Sternberg, R. M., Novick, G. E., Gao, G.-P., Herrera, R. J., *Genetica* 1992, *86*, 215–146.
[29] Makalowski, W., Mitchell, G. A., Labuda, D., *Trends Genet.* 1994, *10*, 188–193.
[30] Smidt, M., Kirsch, I., Ratner, L., *J. Clin. Invest.* 1990, *86*, 1151–1157.
[31] Vidaud, D., Vidaud, M., Bahnak, B. R., Siguret, S. G., Laurin, Y., Meyer, D., Goossens, M., Lavergne, J. M., *Eur. J. Hum. Genet.* 1993, *1*, 30–36.
[32] Daniels, G. R., Deininger, P. L., *Nucleic Acids Res.* 1985, *13*, 8939–8954.
[33] Sawada, I., Schmid, C. W., *J. Mol Biol.* 1986, *192*, 693–703.
[34] Batzer, M. A., Stoneking, M., Alegria-Hartman, M., Bazan, H., Kass, D. H., Shaikh, T. H., Novick, G. E., Ioannou, P. A., Scheer, W. D., Herrera, R. J., Deininger, P. L., *Proc. Natl. Acad. Sci. USA* 1994, *91*, 12288–12292.
[35] Novick, G. E., Menendez, C. M., Novick, C. C., Duncan, G., Yunis, J., Yunis, E., Deininger, P. L., Batzer, M. A., Herrera, R. J., *Int. Pediatrics* 1994, *9*, 60–68.
[36] Batzer, M. A., Deininger, P. L., *Genomics.* 1991, *9*, 481–487.