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Paternity inclusion and exclusion in different types of genetic kinship investigations conducted in a clinical chemistry laboratory of the Federal District (Brazil)

Chianca, CF[1]; Santa Rita, TH[1]; Vasques, JA[1,2]; Abdalla, LF[2]; Costa, SSS[2]; Sousa, CF[2]; Barra, GB[1,2]

[1.] Universidade de Brasília, Brasília, Brasil.
[2.] Laboratório Sabin de Análises Clínicas, Brasília, Brasil.
e-mail: gbbarra@gmail.com

Background: The paternity test is progressively becoming a clinical laboratory test. This analysis is based on comparing at least fifteen short tandem repeat (STR) DNA markers between the child and alleged father, in the presence or absence of the biological mother, which classifies the exam in trio or duo, respectively. The incompatibility in three or more STRs characterizes a paternity exclusion. The compatibility between all regions characterizes a paternity inclusion. The description of the types of cases (duo/trio) and results (inclusion/exclusion) in clinical laboratories are scarce. In this work, these parameters were evaluated retrospectively after eighteen months of implementation of this test in our laboratory. **Methods:** Through the retrospective analysis of our database, we assessed the genetic kinship investigations performed between May 2011 and October 2012. Nine hundred and fourteen investigations were conducted, all involving individuals residing in Brazil's Federal District. The type of case, the conclusion and their distribution over the months were recorded and presented as absolute and/or relative frequency and mean ± standard deviation, when appropriate. The chi-square test was used to compare the obtained ratios. University of Brasília ethical committee approved this study. **Results:** Out of a total of 914 paternity cases, the trios occurred in a higher prevalence compared to duos, 596 (65.2%) versus 318 (34.8%). Moreover, the inclusions were more prevalent than exclusions, 610 (66.7%) versus 304 (33.3%). Besides that, the proportion of inclusion/exclusion were similar between trios and duos, 201 (33.72%) exclusions for trios and 103 (32.39%) exclusions for duos (p=0.68). Furthermore, the inclusion/exclusion and trio/duo proportions remained homogeneous in the eighteen-month studied period, 34.78 ± 6.78% for exclusions (p = 0.45) and 34.72 ± 5.26% for duos (p = 0.97). **Conclusion:** In paternity testing, the trios were more frequent than duo. This result can be explained by the fact that trio has lower complexity analysis than duo, because the alleles not transmited from mother are determined with precision, making it a test cheaper than duo. Furthermore, the paternity inclusion is the most prevalent result type and the inclusion/exclusion proportion observed in this study is similar to that reported by forensic laboratories (32%). Moreover, the homogenity in inclusion/exclusion and trio/duo proportions suggest that these parameters associated with paternity testing remain similar over time.

KEYWORDS: Short tandem repeats (STR), Paternity test, Paternity inclusion, Paternity exclusion, Brazil´s Federal District.

Forensic DNA Brazilian researchers indexed in PUBMED - historical analysis

Kanto, EA[1]; Villhena, JCB [1]; Mendes, GFA [1]; Lopes, RS [1]; Batista, FF[1]

[1.] Universidade Nove de Julho (UNINOVE).
e-mail: evyanzai@gmail.com; evelyn@uninove.br

Introduction: PubMed - US National Library of Medicine, National Institutes of Health, comprises more than 22 million citations for biomedical literature from MEDLINE, life science journals, and online books. Actually is the most international important base of medical articles. **Objective**: This research analyzed the history of Brazilian articles that have relation with forensic genetics or forensic DNA published on Brazil. Method and material: Articles was analyzed from PubMed, using the keywords “forensic DNA Brazil”. **Results**: There are 6376 articles related with forensic DNA indexed from PubMed until February, 2013. The researches of forensic genetics or DNA forensic in Brazil have been released since 1993, according articles indexed from PUBMED - US National Library of Medicine, National Institutes of Health. The first article found, was published in 1992 by American researches, from a case reported in Brazil, related Josef Mengele remains. The most of articles were related with populations studies, alleles or haplotypes frequency (65%) and the others involved different methodologies (35%). This result was equivalent one percent from the articles publishied around the world indexed from PubMed. Conclusion: Brazilian researches involved forensic DNA contributed, but was correspond only a little part of this kind of articles.

KEYWORDS: Forensic DNA; History; Brazilian research.

Analysis of the Y chromosome in the population of the state of Espírito Santo, Brazil, for application to the Human Identification

Ambrosio, IB[1]; Braganholi, DF[1]; Martins, JA[1]; Cicarelli, RMB[1]

[1.]Laboratory of Paternity Investigation, UNESP, Araraquara, SP, Brazil.
e-mail: isabrunelli@hotmail.com

Human identification by DNA analysis uses the genetic profile of an individual studying a combination of markers inherited from their parents. The genetic markers most widely used in routine forensic are present in the autosomes, however, the markers present in sex chromosomes (X and Y) and mitochondrial DNA helps analysis efficiently. Thus, the Y chromosome markers have been widely studied because beyond the forensic field, they have several applications in the field of evolution, such as in the understanding of population genetics and exploitation of human evolutionary history. Two main categories are currently used to examine the Y chromosome: biallelic loci (single nucleotide polymorphisms - SNPs - and Alu insertion) and multiallelic loci (minisatellites and microsatellites - STRs). The Y chromosome has many applications in human identification testing, including forensic analysis in cases of sexual violence or complex paternity cases where the alleged father is absent, because in both situations it is possible to obtain the line. In addition, currently, the yearning for understanding human history and migration patterns over time are responsible for the development of Y chromosome markers mainly in Brazil, since it is known that the Brazilian population is one of the most heterogeneous populations in the world . Due to the potential of genetic markers on the Y chromosome, which has been widely explored by population genetics and forensics, this paper aims to extend the data of the Brazilian population, which are still scarce, analyzing 12 STRs of the Y chromosome using the kit Powerplex Y (Promega) in samples of 250 individuals residing in the state of Espirito Santo, Brazil. The kit proved to be quite efficient for the analysis of individual markers and characterize the samples. The profiles obtained by capillary electrophoresis are being submitted to the database YHRD - Y Chromosome Haplotype Reference Database contributing to the knowledge of the frequencies of these STRs in the Brazilian population allowing further study of the origin patriline of individuals residing in the state of Espirito Santo.

KEYWORDS: Y chromosome; STR´s; Human Identification.

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Padronização de uma PCR espécie-específica para diferenciar amostras de animais para aplicação forense

Fialho, VLS[1]; Britto, KB[1]; Paneto, GG[1]

[1.] Universidade Federal do Espírito Santo, Centro de Ciências Agrárias, Alegre, ES.
e-mail: veronicaluizasilveira@hotmail.com

A Ciência Forense visa auxiliar a investigação criminal utilizando conhecimentos técnico-científicos. Em uma investigação forense, podemos encontrar os mais variados materiais biológicos possíveis. Portanto, é importante conhecer as espécies de origem da amostra. O DNA mitocondrial (DNA mt) é o genoma de escolha para a identificação da espécie. As sequências de nucleotídeos do gene citocromo oxidase (COI), de humanos e animais, são utilizadas por serem conhecidas como específicas de cada uma das espécies. A técnica padrão ouro para sua análise é o sequenciamento do DNA mt, entretanto seu custo é elevado. Sendo assim, novos protocolos alternativos para identificação de espécies são desejáveis para facilitar a sua aplicação na rotina forense. O objetivo deste trabalho foi padronizar uma metodologia de identificação de espécies (humana e não-humana: cães e gatos) utilizando *primers* específicos em PCR multiplex. Para isso, foram desenhados *primers* específicos para cada uma das espécies anteriormente citadas, com amplicons de diferentes tamanhos. Os mesmos foram desenhados à mão, baseados no alinhamento de sequências do DNA mt depositadas no GenBank, utilizando o programa BioEdit. Para análise das temperaturas de anelamento foi utilizado o programa PerlPrimer e para análise de possíveis interações foi utilizado o programa Autodimer. A reação de PCR foi padronizada inicialmente com os pares de *primers* em separados e posteriormente testados em reação multiplex, além disso, foi testada sua especificidade. A avaliação dos produtos de PCR foi feita visualmente, após eletroforese em gel de agarose 1,5%, por 25 minutos a 80 volts, utilizando-se 5 µl do produto da PCR e coloração com brometo de etídio. Os três pares de *primers* (humano, cão, gato) foram testados em separado e posteriormente padronizados em multiplex, utilizando-se, para isso, o DNA extraído de sangue de humanos, cães e gatos. A reação multiplex foi padronizada gerando fragmentos de 122 pares de bases para humano, 225 pb para cão e 260 pb para gato. A diferença de tamanho dos fragmentos possibilita a identificação da espécie. A reação mostrou-se totalmente específica, visto que apenas uma banda foi observada na reação em multiplex quando apenas um tipo de DNA foi utilizado. Os resultados deste projeto poderão ser utilizados na criação de um kit de interesse forense para a rápida identificação de amostras das espécies estudadas, com aplicações nos mais variados materiais biológicos, como sangue, saliva, e principalmente, pelos sem raiz.

PALAVRAS-CHAVE: *PCR* espécie-específica; DNA MT; citocromo oxidase I (COI*)*.

Genetic identity by DNA analysis and legislation in Brazil

Mello, RB[1]*; Iwamura, ESM[1]

[1.]Laboratory of Molecular Pathology, Department of Pathology – Escola Paulista de Medicina/ Universidade Federal de São Paulo – São Paulo, SP – Brasil.
e-mail: rafamello.biomed@gmail.com

The genetic identification of convicted criminals of violent and hideous crimes is a common practice in more than 40 countries worldwide. Very recently, in Brazil, a new law concerning this subject came into effect. The Law nº12.654/2012, sanctioned in May 2012 whose effect came into in the end of the same year, determines as compulsory the genetic identification of persons convicted of violent and hideous crimes. The genetic sample collected will be stored at the National DNA Database, which will assist with the elucidation of violent crimes. The objectives of this review are: a) To present the use of criminal genetic identification in other countries which have adopted this measure and the biotechnological methodology recommended by Interpol for the applications of such analysis; b) To discuss the benefits and misconceptions of the recently created law, the main aspects contained in the legal scope of the recent modification of Brazilian Forensic Genetics, as well as its peculiarities before the mentioned legal enactment. Also, examples of the possible use of the new law in some cases of impact happened in the last decade in Brazil. Data from the National Council of the Public Ministry, 2011, indicate that there are approximately 150.000 surveys of unsolved homicides in the country, data indicating that only 4% of homicide cases in Brazil are solved. Meanwhile, countries in Europe that use DNA databases have a much higher rate (approximately 90%). The Law nº 12.654/2012 represents only the beginning of a new phase in Brazilian Forensic Genetics, still lacking of some refinements, however it is an accomplishment against the impunity perception that endures in the country. Undeniably, the sanction of a new law denotes a major step in the pursuit of the justice, however, it behooves the society and the authorities to discuss and make this step that endure and be effective, preferably successfully.

KEYWORDS: Database; CODIS; Interpol; DNA; Law 12.654/2012.

Relationship between ancestry, human pigmentation and SLC45A2 gene diversity in Brazilian afro-derived communities

Resende, TA[1]; Gontijo, CC[1]; Mendes-Junior, CT[2], Klautau, MN[1], Oliveira, SF[1]

[1]Laboratório de Genética. Depto. Genética e Morfologia, Instituto de Ciências Biológicas, Programa de Pós Graduação em Biologia Animal, Universidade de Brasília-UnB.
[2] Depto. Quimica, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo.
e-mail: tatidosanjos@gmail.com; silviene.oliveira@gmail.com

Criminal investigations often deals with genetics profiles which has no reference, so there is a demand for help to reduce the number of suspects in an investigation. Because of that, for a few years now, there is an interest in the forensic field for genetic markers regarding morphological traits. Previous works have shown low correlation between ancestry and skin pigmentation in admixed countries, like Brazil. However, could skin pigmentation candidate controller genes be related to individual ancestry? If so, individual ancestry could tell the probable features of the offender, reducing time spent during investigation? In this work, association was searched between SLC45A2 SNPs alleles, haplotypes, phenotypic classification and individual index of African ancestry. Six SNPs, located in SLC45A2 gene, were genotyped [rs732740 (intron 1 T>C), rs181832 (intron 2 T>C), rs3756462 (intron 2 T>C), rs26722 (exon 3 T>C), rs16891982 (exon 5 G<C; Leu374Phe), and rs35394 (intron 5 A>G)] in 178 individuals from three Brazilian's *quilombos* – Kalunga (Goias), Mocambo (Sergipe) and Sacutiaba (Bahia). Genotypes were obtained by PCR-RFLP and allele-specific PCR, followed by 10% vertical polyacrylamide gel electrophoresis, stained with silver nitrate. Individuals were classified by interviewer according to skin color in white, black, and mixed. Individual African ancestry index estimates were obtained using Structure 2.3.3 software with individual genotypes for 107 Ancestry Informative Markers (AIMs) obtained from three multiplex. SNPStats software was employed to evaluate associations between SLC45A2 SNPs genotypes and Individual African ancestry index and also between SLC45A2 SNPs genotypes and classification. African ancestry index ranged from 10 to 80% in sample. Significant association was verified between African ancestry index and some of the SNPs genotypes, and also with haplotypes. Color classification was associated with some genotypes of all SNPs, except rs732740 (monomorphic). Results suggest that SLC45A2 SNPs genotyping can be useful to signalize individual ancestry as well as individual skin color appearance.

KEYWORDS: SLC45A2 gene; individual ancestry; association analysis; Brazilian quilombos, skin color.

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The importance of lineage markers to fortify the hypothesis of founder effect of E180Splice mutation in GHR gene causing Laron syndrome: a witness of Jewish exodus

Gonçalves, FT[1]; Pinto, EM[10]; Laron, Z[2]; Guevara-Aguirre, J[3]; Carsola, FG[4]; Damiani, D[5]; Lins, TS[6]; Shevah, O[2]; V, Hwa[7]; Rosenbloom, AL[8]; Rosenfeld, RG[9]; Arnhold, IJP[10]; Jorge, AAL[10]; Fridman, C[1]

[1.] Department of Legal Medicine, Ethics and Occupational Health, Medical School, University of São Paulo, São Paulo, Brazil.
[2.] Schneider Childrens' Med Ctr, Petah-Tikva, Israel.
[3.] Inst for Endocrinology Metabolism and Reproduction, Quito, Ecuador.
[4.] University of Chile, Santiago, Chile.
[5.] Child Institute , Medical School, University of São Paulo, São Paulo, Brazil.
[6.] Inst Materno Infantil de Pernambuco, Recife, Brazil.
[7.] Oregon Hlth and Scis University, Portland, OR.
[8.] University of Florida Coll of Med, Gainesville, FL.
[9.] Lucile Packard Foundation for Children's Hlth, Palo Alto, CA.
[10.] Department of Endocrinology, Medical School, University of São Paulo, São Paulo, Brazil.
e-mail: fernandatoledo@usp.br

Laron syndrome (LS) is a genetic disorder caused by mutations in the Growth Hormone Receptor (*GHR*) gene leading to severe GH insensitivity. The most prevalent *GHR* mutation is E180splice which was found only in isolated population of Spanish descent from the Andes (Ecuador); recently it was also found in Israel, Brazil and Chile. A common origin of the E180splice mutation is postulated and it is supported by the presence of common introns haplotype. Herein, to confirm this hypothesis, we analyzed polymorphic markers surround GHR gene (markers from chromosome 5 short arm, intragenic SNPs and GHR exon 3), paternal lineage origin analyzing Y-chromosome STR and maternal lineage investigating mitochondrial DNA haplogroups. We genotyped 20 LS patients from Ecuador, Israel, Brazil and Chile which were homozygous for E180splice mutation and their first degree relatives heterozygous for mutation and compared to 42 normal individuals and 5 LS patients with other mutations. An identical haplotype was identified in all but one of the patients carrying the E180splice mutation: D5S2082: 192/192; D5S665: 150/150; D5S2087: 246/246; rs6179 G/G rs6180 C/C, IVS9 haplotype-I and GHRfl. One patient differed from others by D5S2082 (168/192). Control individuals and LS patients with other mutations showed different haplotypes. This result corroborated that the E180splice-associated haplotype found in all families has not independent origins ($p < 10^{-8}$) and they have a common ancestor. Half of patients showed paternal origin belonging to haplogroup R1b (found in Portugal and Spanish) and 46.6% belonged to haplogroups J and E (typical in the Middle East and Eastern European). mtDNA analysis showed that 29.4% of patients belonged to Amerindian haplogroup B, 17.6% had European haplogroup J (from Balkan Peninsula), 35.3% presented African haplotypes and the Israeli patient showed subhaplogroup R0 found in Arabia. Jews were expelled from Spain in 1492, but many had converted to the Christian faith. These New Christians emigrated from the Iberian Peninsula to the New World. The founder effect of E180Splice mutation may be explained by this historic event, which we reinforced with strong evidence of co-segregation between several polymorphisms, the germline E180splice mutation and paternal lineage in LS patients from different countries, indicating this mutation originated from a single common ancestor.

KEYWORDS: Laron syndrome; Lineage origin; Y-chromosome STR; Mitochondrial DNA; Jewish population.

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Analysis of human mitochondrial DNA hypervariable region (HVR1) polymorphisms in a population from Santa Catarina State, Southern Brazil

Torres, SRR[1,2]; Uehara, CJS[1,3]; Sauerbier, TS[1]; Muniz, YCN[2]; Marrero, A[2]; Souza, IR[2]

[1.] Instituto Geral de Perícias de Santa Catarina, SSP, Florianópolis, SC.
[2.] Universidade Federal de Santa Catarina, Florianópolis, SC.
[3.] Universidade Federal do Paraná, Curitiba, PR.
e-mail: rachadeltorres@gmail.com

The State of Santa Catarina, located in southern Brazil, had an occupation process mediated by several migratory waves coming from Portugal, Germany, Italy, Spain, Belgium, Poland, and Africa; besides the Amerindians that already lived in the region. This process resulted in the formation of a heterogeneous population and was initiated by "Vicentistas" in the seventeenth century, continued with the Azorean community and Natives of São Paulo State in the eighteenth century, culminating with the arrival of non-portuguese european immigrants in the nineteenth and twentieth centuries'. According to the IBGE (the Brazilian Statistics Bureau), the population of Santa Catarina consists of 6,248,436 inhabitants, which is composed of 88.1% White, 9.0% Brown (mixed race), 2.7% of Black and 0.2% Yellow or Amerindian (data based on self-report questionnaire). The sequencing of mitochondrial DNA (mtDNA) has been used to identify variability in populations. Thus the aims of this project were to genetically characterize the variability of matrilineal population of Santa Catarina by sequencing the hypervariable region of mtDNA (HVR1), as well as the building of a database, and standardizing a technique that can assist in forensic cases, involving degraded and/or insignificant samples. DNA extractions were performed in 118 unrelated individuals using FTA Reagent, while HVR1 was amplified by PCR reaction and sequenced on both DNA strands using the BigDye v3.1 chemistry protocol. Subsequently, the samples were submitted to capillary electrophoresis in ABI 3130 genetic analyzer. The obtained sequences were aligned and compared with the revised Cambridge Reference Sequence (rCRS) using Bioedit package, and phylogenetic relationships were analyzed with Mega software. The determinations of haplogroups were made by identification of characteristic mutations with the aid of the mtDNAmanager database. In the analysis of the population of Santa Catarina were identified 21 and 57 distinct haplogroups and haplotypes, respectively. The results showed a greater contribution of haplogroup H (22.8%), followed by haplogroups C (15%), A (8.5%) and K (4.3%). We identified 52.6% of European haplogroups (H, J, K, U, T, W), 27.6% of Native American haplogroups (A, B, C and D) and 5.8% of African haplogroups (L). However, 14% of the haplogroups were not identified. The haplogroups identified showed the coexistence of three maternal lineages with different phylogeographic origins. These results led to the expansion of genetic data disposable for Santa Catarina population and the implementation and standardization of mtDNA analysis for application in forensic cases in the State.

KEYWORDS: mtDNA; HVR1; Haplogroup.

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Y-Chromosomal STR haplotypes in a sample from Brasília, Federal District, Brazil

Che, G[1]; Freitas, JM; Badaraco, JL[1]; Rosa, LS[1]; Martins, AD[1]; Jacques, GS[1]; Paranaíba, RTF[1]

[1.] Laboratório de Genética Forense, Instituto Nacional de Criminalística, Diretoria Técnico Científica, Departamento de Polícia Federal, SAIS Quadra 07 Lote 23, CEP 70610-200, Brasília, DF, Brazil.
e-mail: che.gc@dpf.gov.br

Brasília is the federal capital of Brazil. Built from scratch in the late 1950´s, it attracted migrants from different regions of Brazil, mostly from Central West, Northeast and Southeast. Due to its formation, its population is admixed. The haplotypes of seventeen Y-chromosome STR loci (DYS19, DYS385I/II, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and Y GATA H4) were determined in a sample of 300 blood donors from Brasília. A total of 290 different haplotypes were identified, 280 of which were unique. The haplotype diversity (HD) was calculated as 0.9965. Diversity indices obtained for each locus ranged from 0.493 to 0.891, with an average of 0.657. Haplotype diversity and diversity indices were similar to those obtained for Brazilian populations in previous studies. Haplotypes with double peaks at DYS391, DYS439, DYS635, besides those at the DYS385I/II locus were observed. Moreover, tri-allelic patterns and a null allele were observed at the DYS385I/II locus. Pairwise population comparisons based on R_{st} with other Brazilian populations showed no significant differences. Estimation of Y Chromosome STR haplotype frequencies is very important for kinship and casework analysis when this genetic marker is used.

KEYWORDS: Y-STRs, Brasília, haplotype.

Resolving cases of paternity with degraded DNA samples

Braganholi, DF[1]; Ambrosio, IB[1]; Alves, RW[1]; Martins, JA[1]; Cicarelli, RMB[1]

[1.] Laboratory of Paternity Investigation, UNESP, Araraquara, SP, Brazil.
e-mail: danilobraganholi@hotmail.com

Analysis of DNA polymorphisms is the best tool for resolving cases of paternity investigation and human identification where the STR (short tandem repeat) markers used in autosomal regions are the main and most used for this purpose. However in specific cases, only these markers are not sufficient to generate an appropriate resolution with accurate and reliable result, so it is also used STRs analysis of the sex chromosomes X and Y. Despite the incontestable reproducibility of these markers, when these are analyzed in degraded or contaminated samples may not produce good results, which complicate the resolution in most cases. Thus there is a need for alternative and currently is the most appropriate use of insertin-deletion polymorphisms (INDELs). An agreement was signed in 2011 between the Laboratory of Paternity Investigation of UNESP and Public Defender of the State of São Paulo to meet the demand of the state since this is currently the only laboratory performing paternity tests with samples from exhumation for free justice. We performed the cases for analysis of STRs by using the Powerplex 21system kit (Promega) and increase the discriminatory power for analyze of the 30 polymorphisms INDELs using the Investigator DIPplex kit (QIAGEN) for to solve cases with degraded DNA samples, evaluating the contribution of these resolutions for INDELs. Conducted during the 2012 year 12 cases with some sample from exhumation and, in 10 not necessary to analyze INDELs because STRs were sufficient to obtain conclusive results. In 2 cases, only the autosomal STRs were not sufficient and analyzed the INDELs and in 1 case was used the kit PowerPlex Y (Promega). The results of these analyzes have provided an increase in power of discrimination and in the case of the INDELs also give better quality profiles with electrophoresis, therefore, the fragments amplified by PCR are smaller in comparison to STR, which assists in the amplification of sample with a degraded DNA, and therefore a interesting tool for solving these cases.

KEYWORDS: INDELs; STRs; Paternity.

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Uniparental markers of ancestry in usual prevalence areas of cleft lip with or without cleft palate identified by ECLAMC in Argentina

Miyoshi, MTC[1,2]; Vieira-Machado, CD[1,2]; Bailliet, G[4]; Castilla, EE[1,3]; Orioli, IM[1,2]

[1] Instituto Nacional de Genética Médica Populacional (INAGEMP), Rio de Janeiro, Brasil.
[2] ECLAMC, Departamento de Genética, Instituto de Biologia, UFRJ, Rio de Janeiro, Brasil.
[3] Laboratório de Epidemiologia de Malformações Congênitas, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil.
[4] ECLAMC, Instituto Multidisciplinario de Biología Celular (IMBICE), CCT La Plata-CONICET, 1900 La Plata, Argentina.
e-mail: maluahtc@gmail.com

Isolated cleft lip with or without cleft palate (CL/P) is a common birth defect that has a prevalence of 1.1/1000 live births in South America. A higher prevalence is seen in North American Indians (3.6/1000 births), and it progressively declines for Asians (2.1/1000 births in Japanese and 1.7/1000 births in Chinese), Caucasians (1.0/1000 births), and Africans (0.3/1000 births). In a previous study from the ECLAMC (Latin American Collaborative Study of Congenital Malformations) high prevalence clusters of CL/P were identified in hospitals from Argentina, Brazil, Chile, Bolivia, Ecuador, and Colombia. There were also low prevalence clusters of CL/P in Venezuela and Uruguay. Outside those clusters there were hospitals with usual CL/P prevalence in Brazil, Argentina, Colombia, Chile, Venezuela, Paraguay, and Peru. Also, in a mitochondrial haplogroup study performed in our laboratory using ECLAMC samples from high prevalence clusters for CL/P, the relative risk for CL/P was 2.9 times higher (CI 95%: 1,63 - 5,25) in Amerindian population than in non-Amerindians. In the same population, the relative risk for CL/P using the Y chromosome marker DYS199 was 2.4 times higher (CI 95%: 1,65 - 3,39) in Amerindian population than in non-Amerindians. The main objective of this study is to determine the frequency of Amerindian mitochondrial haplogroups, (A, B, C and D), and of the Y chromosome allele, DYS199T, among ECLAMC cases of CL/P and their controls in hospitals with usual prevalence of CL/P. The prevalence of Amerindian uniparental markers was compared between CL/P cases and their controls. Until now we have analyzed 78 cases and 91 controls from ECLAMC Argentinian hospitals (Buenos Aires, Rosario, Cordoba, Mendoza, San Martin, San Luis, San Salvador). The obtained results were also compared with high CL/P prevalence clusters. There was no statistically significant difference between CL/P cases (77.5%) and controls (84.6%) for Amerindian mtDNA haplogroups in the usual prevalence areas of CL/P in Argentina. This result could be due to the small sample sizes or to the small CL/P relative risk for Amerindians in the usual CL/P prevalence areas. However, there was statistically significant difference between cases from CL/P usual prevalence areas (78.2%), and cases from high CL/P prevalence areas (93%) (χ^2_y = 15,598; GL = 1; P < 0,0001) for Amerindian mtDNA haplogroups. There was statistically significant difference between cases (43.35%) and controls (26.76%) for DYS199T (χ^2_y = 19,483; GL = 1; P < 0,0001) in the high prevalence areas of CL/P, but DYS199T was not until now observed in 27 cases or 16 in controls from the usual prevalence areas for CL/P. With the actual sample size we could not demonstrate higher uniparental Amerindian ancestry in the CL/P patients than in controls from usual prevalence areas of CL/P.

KEYWORDS: Oral cleft; Cluster; Ancestry, Amerindian; ECLAMC.

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Allele frequencies for fifteen autosomal short tandem repeats in a population sample from Federal District (Brazil) – a territory that arose from nothing to the reality

Santa Rita, TH[1]; Chianca, CF[1]; Abdalla, LF[2]; Costa, SSS[2]; Velasco,LFR[2]; Sousa, CF[2]; Barra, GB[1,2]

[1.] Universidade de Brasília, Brasília, Brasil.
[2.] Laboratório Sabin de Análises Clínicas, Brasília, Brasil.
e-mail: gbbarra@gmail.com

Background: Brazil's Federal District and Brasília, the current federal capital, were founded in April 21, 1960 from the desire to transfer the federal capital from Rio de Janeiro to the center of the country. In 2010, the Federal District population is comprised of 2.562.963 inhabitants, 54% of people were born locally and the 46% were immigrants from other Brazilian macroregions: 2% North, 23% Northeast, 6% Central West, 13% Southeast, and 2% South. Paternity testing is progressively becoming a clinical laboratory test. This analysis is based on whether or not short-tandem-repeat alleles (STR) are shared between the child and the alleged father. If paternity inclusion is found there are two possibilities: the alleged father is the biological father or, due to chance, he has an identical STR profile of the true biological father. The possibility of two unrelated individuals possessing identical STR profiles can be determined statistically (Random match probability). Preferably, these calculations should be done with local population allele frequencies. In this work, we investigated the allele frequencies and the forensic efficiency parameters of a fifteen autosomal STR loci in Brazil's Federal District population. **Methods:** Through the retrospective analysis of our laboratory results database, we retrieved the STR profiles of 462 unrelated adults involved in paternity testing performed between May 2011 and October 2012. All profiles where from individuals residing in Brazil's Federal District. The allele frequencies, Hardy–Weinberg equilibrium deviation, polymorphism information content, power of discrimination, power of exclusion, matching probability, typical paternity index, and Nei's genetic distances to other Brazilian macroregions populations were calculated for the fifteen autosomal STRs included in AmpFlSTR® NGM™ (Applied Biosystems). University of Brasília ethical committee approved this study. **Results:** No significant deviation from Hardy–Weinberg expectations were found. For all STRs, the combined matching probability was 4.85×10^{-20}, the combined power of discrimination was 0.99999999999999999951, and the combined power of exclusion was 0.9999998. The highest typical paternity index was for both D2S1338 and FGA (4.0526) and the lower was for D22S1045 (2.0263). Nei's Genetic distances showed that Federal District allele frequency was grouped in a cluster composed by Southeast, Northeast and Brazil overall populations. Moreover, Federal District assumes an intermediate position relative to south, and it is quite distant from North and even from the Central West. **Conclusion:** The fifteen studied STR loci are a highly informative genetic system for Brazil's Federal District population, the probability that two randomly selected individuals have exactly the same STR profile is 4.85×10^{-20}. Moreover, the genetic distances found were appropriate to the population composition reported in the 2010 National Survey Inquiries, in which the main Federal District immigrants proportion were from Northeast and Southeast. The alleles frequencies described here will be used in routine casework.

KEYWORDS: Short tandem repeats (STR); Federal District population; Forensic genetics.

A importância dos Short Tandem Repeats do cromossomo Y(STRs-Y)e suas implicações na prática forense. O que há de novo no Brasil?

Zanta, NC[1]; Martinez, D[1]; Soares-Vieira, JA[2]; Iwamura, ESM[1]

[1.] Laboratório de Patologia Molecular – Escola Paulista de Medicina - Universidade Federal de São Paulo – São Paulo, SP – Brasil.
[2.] Departamento de Medicina Legal, Ética Médica e Medicina Social e do Trabalho – Faculdade de Medicina da Universidade de São Paulo – São Paulo, SP – Brasil.
e-mail: nataliaczanta@gmail.com

De acordo com o Código Penal Brasileiro,em seu Artigo 213 (Redação dada pela Lei nº 12.015, de 2009), estupro é definido como "constranger alguém, mediante violência ou grave ameaça, a ter conjunção carnal, ou praticar ou permitir que com ele se pratique outro ato libidinoso". Hoje, a pena para o crime de estupro no Brasil é de 6 a 10 anos de reclusão para o criminoso, aumentando para 8 a 12 anos se há lesão corporal da vítima ou se a vítima possui entre 14 e 18 anos de idade; e para 12 a 30 anos, se a conduta resulta em morte. Em todo o Estado de São Paulo, de 2000 a 2009, segundo a Secretaria de Segurança Pública (SSP), foram registrados 22.702 casos de atentado violento ao pudor e 18.770 de estupro. Com a unificação desses dois crimes, de 2009 até o segundo trimestre de 2012, foram 26.483 ocorrências de estupro. Estudos do Ministério da Saúde sobre tal assunto ainda indicam que a maioria dos casos no Brasil não é sequer relatada às autoridades policiais, por causa dos aspectos morais, sociais e emocionais envolvendo a vítima. Tendo em vista essa situação, a utilização dos STRs do cromossomo Y têm se mostrado muito útil em laboratórios forenses nas perícias que envolvem agressão sexual. Nesses casos as evidências, como a secreção vaginal, irão conter tanto DNA feminino, quanto masculino, sendo o primeiro mais abundante. Isso não é um problema quando se estuda os marcadores do cromossomo Y, pois o DNA feminino presente na amostra não vai interferir na amplificação por PCR. Outra vantagem é que devido à natureza haplóide do cromossomo Y, os perfis genéticos podem ser definidos nos casos onde mais de um agressor participou do crime. A coleta de perfil genético para identificação criminal, prevista na Lei 12.654/2012, entrou em vigor em 29/11/2012. O banco de DNA ajudará na elucidação de delitos nos quais forem encontrados vestígios com materiais biológicos de criminosos. Diante disso, faz-se mistér uma discussão mais aprofundada sobre as amostras biológicas oriundas de crimes sexuais e suas implicações na prática forense. O presente trabalho discute os estudos realizados no Brasil envolvendo os STRs do cromossomo Y.

PALAVRAS-CHAVE: Codigo Penal; Str Y; Crimes Sexuais; Legislação.

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Efficiency of STR analysis in FFPE samples for human identification purposes

Funabashi, KS[1]; Barcelos, D[1]; Sousa, MLAPO[1,2]; Franco MF[1]; Visoná, I[1] Iwamura, ESM[1]

[1.] Molecular Pathology Laboratory, Department of Pathology – Escola Paulista de Medicina/ Universidade Federal de São Paulo – São Paulo, SP – Brasil.
[2.] Núcleo de Biologia e Bioquimica - Instituto de Criminalística de São Paulo – Superintendência da Polícia Técnico Científica de São Paulo/SPTC SP.
e-mail: eiwamura@gmail.com

Archived biopsy or *post-mortem* formalin-fixed paraffin-embedded (FFPE) tissues are potential reference samples for genetic testing for forensic applications, such as identification of unknown corpses in mass disasters or DNA paternity testing cases in which the putative father is not available. The FFPE could be the ultimate DNA resource available for genetic analysis. However, the DNA extracted from these samples could be in low amount (depending on the nature and quantity of the tissue included) or with poor quality and fragmented to various extents, due to the fixing and inclusion conditions, as well time and storage conditions**. The aim** of this study is to evaluate the PCR reaction in tissues FFPE using different kits for human identification to investigate the validity of short tandem repeat (STR) analysis in these samples and its potential effect on human identification. **Material and Methods**: Autopsy healthy tissues of FFPE spleen and liver, from the same individuals (n=10) were compared for DNA analysis after PCR amplification. For each paraffin block, three 10-µm-thick sections were cut and collected in a 1.5-ml microtube. Frozen tissue was used as a control sample. DNA was extracted using commercial kit (QIAamp DNA Mini kit Qiagen). DNA amplification was performed using two different amplification kits: AmpFlSTR® Identifiler™ (Applied Biosystem) and PowerPlex16™ (Promega) PCR Amplification Kits following the manufacturers' recommendations. PCR reaction was conducted using the GeneAmp® PCR System 9700. The amplified alleles were separated by capillary electrophoresis using ABI PRISM 3130 Genetic Analyzer. The sizes of the PCR products were evaluated by the software GeneMapper 3.2 using allelic ladders as a comparison. **Results and discussion**: The quality of DNA extracted from FFPE often complicates the analysis or limits its efficiency. Many studies have demonstrated that formaldehyde (H_2CO), the main constituent of formalin, is responsible for DNA molecules cross-linking reactions and fragmentation. Profiles obtained using the PowerPlex16™ system were generally incomplete, only small fragments less than 200bp being amplified, with higher levels of instability than those obtained using Identifilfer™. The success rate of amplification was 64.3% for the Identifiler™ against 62.8% for PowerPlex16™. Both kits have 16 genetic markers, among the PowerPlex16 markers, eight are smaller than 250 bp and seven are between 250bp to-400bp, for Identifiler, nine are smaller than 250 bp. This preliminary results has been established because some difficulties can arise when the authenticity of the reference sample is questionable, or when the sample is taken from tissues with possible genetic variability, such as a malignant tumor tissues. Other kits (Minifiler™ and PowerPlex® ESI 17 System) has been also tested in this FFPE samples.

KEYWORDS: Molecular pathology; Formalin-fixed; Paraffin-embedded; PCR; STR.

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InDel markers for paternity investigation

Santos, MD[1]; Simões, AL[1]

[1.] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, USP, Ribeirão Preto, SP.
e-mail: mar.dmb@usp.br, alsimoes@fmrp.usp.br

In many situations, DNA presented for analysis of genetic markers is fragmented. In these cases, it is necessary to use markers detectable by analysis of small fragments, such as those of insertion/deletion (*InDel*) polymorphisms. Besides this, many other aspects make these markers as chosen in various DNA forensic tests purposes: low mutation rate, polymorphism derives from a single mutational event, *InDels* are spread throughout human genome and can be identified by simple laboratorial analysis (conventional Polymerase Chain Reaction). Furthermore, many of them are Ancestry Informative Markers (AIMs), due to the large frequency difference between continental populations. Large size of some inserts hinders the completion of *insertion* allele synthesis in PCR. To overcome this difficulty, besides the PCR led by pair of primers complementary to insertion flanking regions, it is proposed here a second PCR with one of these primers and another one specific, complementary to insertion segment. For this, we describe trios of primers whose combined use in two reactions easily identifies all three phenotypes of biallelic *InDel* locus. This system has the advantage of producing reduced size amplicons, suitable for fragmented DNA examinations. Three *InDel* loci were chosen for this pilot study (MID1386, MID 818, and MID856) for which were drawn three trios of primers. Genotyping blood samples from 150 pairs of mothers and their newborns allowed confirmation of only two polymorphic forms existing (insertion or deletion), as well *insertion* allele frequency estimation: 0.7878, 0.6486, and 0.2868, respectively, MID1386, MID818, and MID856. Increasing number of these markers will allow its use in identifying cases in which DNA is heavily degraded. Since DNA of fetal origin is fragmented in maternal plasma, another potential use of this technique described corresponds to identify the fetal *'insertion'* allele by fetal DNA analysis from maternal blood samples, featuring a genetic identification examination in the pre-natal phase, but not invasive.

KEYWORDS: Polymorphism; Insertion/deletion; Human identification.

Contribution of the human identification by DNA in the rights of the family: one year of agreement between UNESP/Laboratory of Paternity and the Public Defender Service of the State of São Paulo

Martins, JA[1]; Braganholi, DF[1]; Felix, JHP[1]; de Almeida, CF[1]; Alvarenga, VLS[1]; Cicarelli, RMB[1]

[1.] UNESP - São Paulo State University, School of Pharmaceutical Sciences, Laboratory of Paternity, Araraquara, São Paulo, Brazil.
e-mail: joyce_apa@yahoo.com.br

The right to paternity is consecrated in the Universal Declaration of Human Rights; however, there is a high number of people with incomplete birth registration data in Brazil. The UNESP Paternity Investigation Laboratory started its activities in March 2001 and recently, in October 2011, signed an agreement with the Public Defender Service of the State of São Paulo (DPESP). The current agreement proposes free DNA tests for individuals entitled to legal assistance in Araraquara region (Headquarters) and the courts of municipalities within 100 kilometers of Araraquara, also establishes the maximum of 100 examinations per month of live individuals (theme of this abstract) and five exams of exhumed bodies. The laboratory examines different biological materials such as blood, saliva, hair and bones and offers different techniques for the analysis of DNA including the typing of 21 autosomal (AS) short tandem repeat (STR) markers and, when necessary, 12 Y-STRs, 10 X-STRs and mitochondrial DNA sequences. The methodology and reagents utilized in the examination meet the consensual standards recommended for the identification of humans. At the end of each month, a list of the services provided is sent to DPESP that makes the payment. The request for DNA testing began in December 2011 and until December 2012 were performed 196 examinations involving the typing of 605 samples of living people. These investigations included 165 complete triple (mother, son and supposed father), nine duo (child and alleged father), twelve cases of reconstruction, four cases with mother, son, father registry and supposed father, six cases involving mother, two sons and supposed father, and one case with alleged mother. For reconstruction, six cases were solved by use of AS and X STR markers and others six by AS and Y STR markers. Non-exclusion of the investigated biological relationship was obtained in 130 of 196 cases. A total of seven paternal mutations of one step were found, three to D18S51 marker and one to FGA, D8S1179, D2S1338 and D12S391. In conclusion, the UNESP Paternity Investigation Laboratory has a university outreach program with services provided to the community, provides the resources to educate highly qualified professionals with a team including one PhD (CAPES), 2 academic support and extension research fellows (BAAE II), one specialist student (FUNDECIF), one specialist and one coordinating professor.

KEYWORDS: Paternity testing; DNA; Human identification; Public Defender Service; UNESP Paternity Laboratory.

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Avaliação da eficiência de protocolos para amplificação de DNA mitocondrial por PCR direta em amostras humanas

Britto, KB[1]; Fialho, VLS[1]; Paneto, GG[1]

[1.] Universidade Federal do Espírito Santo, CCA, Alegre, ES.
e-mail: karolbbritto@hotmail.com

O DNA mitocondrial (DNA mt) é encontrado na matriz da mitocôndria e muito se difere do DNA nuclear. Uma única célula possui mais de 5.000 cópias de DNA mt. Existem muitos métodos de extração de DNA, mas a maioria deles contribui para uma perda significativa de DNA, devido às inúmeras transferências de tubos. Isto pode ter um efeito significativo sobre a quantidade do DNA gerado. Uma alternativa à técnica convencional de extração de DNA seguida de amplificação por PCR é o método conhecido como PCR direta, pois poupa o tempo e reagentes da extração do DNA e reduz a perda de amostras com pouco DNA. Assim, novos métodos, mais simples e baratos, para a PCR direta podem permitir sua utilização rotineira na prática forense. Neste contexto, o objetivo deste trabalho foi testar diferentes protocolos para amplificação de DNA mt por PCR direta. Foram testados os seguintes protocolos: (1) Inserção direta das amostras na PCR; (2) Tratamento prévio das amostras utilizando uma solução de lise; (3) Extração de DNA convencional utilizando resina Chelex. As amostras biológicas: células bucais, sangue, e cabelo com raiz, foram coletadas do próprio pesquisador para análise. O gene escolhido foi o Citocromo Oxidase I (COI) do DNA mt. Os produtos amplificados foram confirmados por eletroforese em gel de agarose 1,2%, e comparados com um *ladder*. Todas as amostras foram amplificadas quando utilizado o método convencional de extração de DNA, usando resina Chelex. O tratamento prévio das amostras com a solução de lise, no entanto, se mostrou eficiente para análise do DNA mt das amostras de células bucais e cabelo, entretanto, não foi possível a amplificação em amostras de sangue. Isto se deve, provavelmente, à presença do grupo heme ainda na reação, mesmo após o tratamento. A inserção direta das amostras biológicas na PCR não resultou em amplificação. Substâncias conhecidas como 'inibidores' podem interferir ou previr que o processo de amplificação do DNA aconteça corretamente, afetando assim a amplificação por PCR, dentre eles podemos citar o (a): heme (sangue), melanina (tecidos e cabelo), colágeno (tecido), mioglobina (tecido muscular), polissacarídeos (fezes e material vegetal), sais biliares (fezes), ureia (urina), dentre outros. Com os resultados deste trabalho podemos concluir que é possível amplificar o DNA mt, com o tratamento prévio, de alguns tipos de amostras humanas sem que haja uma extração convencional. Entretanto, novos métodos mais eficientes e rápidos necessitam ser desenvolvidos para viabilizar seu uso na prática forense.

PALAVRAS-CHAVE: PCR direta; DNA mt; Extração.

Utilização de processos eletrônicos para gerenciar amostras e informações periciais DO IML, IC e Labforense na produção de laudo digital de DNA

Francez, PA[1]; Costa, GSMB[1]; Faro, JMF[1]

[1.] Laboratório de Genética Forense da Politec-AP.
e-mail: pabdon@uol.com.br

Os exames de Genética Forense na maioria das vezes necessitam de informações e amostras biológicas provenientes de diferentes institutos. Uma boa interação entre o setor de investigação da polícia civil, os Institutos de Medicina Legal, Criminalística, Laboratório Forense e Laboratório de DNA é fundamental para o sucesso dos exames de Genética Forense. Os exames de DNA em casos de violência sexual, identificação de cadáver ou de amostras coletadas em local de crime, exigem uma complexa cadeia de interações que começa com o atendimento, orientação e encaminhamento das vítimas, familiares e suspeitos para a perícia. As vítimas atendidas nos IMLs devem ter tanto as amostras questionadas quanto as amostras de referência coletadas, identificadas, acondicionadas corretamente e encaminhados para o Laboratório Forense, assim como as amostras questionadas coletadas nos locais de crime. No Laboratório Forense as amostras questionadas devem ser avaliadas em relação à presença e natureza do material biológico identificado e tanto as amostras questionadas quanto de referência dos suspeitos, vítimas e familiares devem ser encaminhadas para o Laboratório de DNA que procedera a realização dos exames de confronto genético. Este fluxo de amostras e informações entre diferentes institutos se torna cada vez mais complexo na medida em que o número de ocorrências atendidas cresce e a demanda por exames de DNA se eleva no Brasil, sendo fundamental a utilização de processos eletrônicos que permitam gerenciar as informações, identificando e vinculando pessoas e amostras provenientes de diferentes setores, permitindo acesso rápido a estas informações, reduzindo a redundância e inconsistência das informações, minimizando o retrabalho, aumentando a segurança e garantindo e registrando a cadeia de custódia de acessos às informações através de identificação digital. Tendo em vista a necessidade de modernizar a troca de informações e amostras entre os diferentes setores da POLITEC-AP, em 2012 deu-se início à Informatização dos Processos e emissão de Laudo Digital nesta instituição, utilizando um sistema baseado em softwares livres, que emprega Banco de Dados PostgreSQL, utiliza plataforma web (funciona em rede local ou na internet) compatível com as tecnologias de assinatura digital BPM (Business Process Manager) e gerenciamento eletrônico de documentos com ferramenta de Workflow integrada. Atualmente o Sistema de Processos Eletrônicos implantadas na POLIEC-AP já abrange mais de 95% de todos os documentos que ingressam na instituição através de um protocolo eletrônico, registra 100% dos acionamentos realizados via setor de comunicação da unidade sede em Macapá e acompanha estes processos até a sua saída através da central de laudos, possui um banco de dados que registra, incluindo imagens digitais, todos os cadáveres e grande parte das amostras e objetos que ingressaram na POLITEC-AP a partir de 2013. Mais de 70% dos laudos emitidos atualmente contam com arquivamento em meio digital, incluindo levantamento fotográfico.

PALAVRAS-CHAVE: Genética forense; Informatização; Processos eletrônicos; Laudo digital.

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Comparison of SNPs and indels as markers used in forensic casework and relationship testing

Whittle, MR[1]; Favaro, EC[1]; Sumita, DR[1]

[1.] Genomic Engenharia Molecular, São Paulo, SP.
e-mail: mwhittle@genomic.com.br

Human identification and relationship testing are typically performed by studying polymorphic genetic markers distributed along the autosomal chromosomes. The loci most used at present are short tandem repeat (STR) multiallelic loci which usually provide sufficient information for conclusions to be reached in casework. In addition to these markers, single nucleotide polymorphisms (SNPs) and deletion-insertion polymorphisms (DIPs or indels) can also be used as an auxiliary tool leading to the resolution of more difficult cases. The advantage of these latter two marker systems in relation to STRs is their low mutation rate and the fact that it is possible to work with smaller amplicons, enabling the study of degraded samples. However, because they are biallelic it is necessary to analyze a larger number of these markers to obtain equivalent strong results. The aim of this study was to compare the performance of indels with those of SNP markers in relationship testing and in some forensic cases. In addition, the genotyping methods were evaluated for each system. Forty autosomal indels were selected from the Marshfield Institute database and genotyping was performed by PCR using fluorescent universal primers. Likewise, 40 autosomal SNPs were chosen from the SNP*for*ID consortium and from the Kidd laboratory and were analyzed using the SNaPshot technique (Applied Biosystems). One hundred trios showing exclusion of paternity and 100 trios showing inclusion of paternity were genotyped using each of the two marker systems. The number of genetic inconsistencies and the combined paternity indices were calculated and compared. Although both systems comprise biallelic markers, we observed that the selected SNPs showed similar or slightly better paternity indices than those seen for the selected indels. Furthermore the SNP panel proved to be more robust when dealing with degraded samples having known PCR inhibitors. However, genotyping of the SNPs is more onerous and more costly than that for the indels. The method using the universal fluorescent primers proved easy to utilise and to be automated, with lower costs. Both the SNP and indel panels proved to be extremely useful complementary tools when employed together with STR markers, both in extended relationship testing and in forensic casework.

KEYWORDS: Human identification; SNP; Indel; Parentage testing; Forensic genetics.

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Dissertação de mestrado

Histopathological study and DNA analysis feasibility in human bones exposed to taphonomic conditions: applications in forensic identification

Soler, MP[1]; Alves, MTS[1]; Iwamura, ESM[1]

[1.] Laboratory of Molecular Pathology, Department of Pathology – Escola Paulista de Medicina, Universidade Federal de São Paulo, Brazil.
e-mail: mipsoler@gmail.com

Brazil has the 6th highest homicide rate in the world and there are many cases in which *post-mortem* human bones that were exposed to adverse environmental conditions are the only materials available for identification. Acid soil and humid tropical climate with high rainfall and temperature can exert direct influence on bone tissue and its cells and these changes in bone microstructure may possibly explain the poor results obtained from these samples concerning DNA analysis in human identification. **The objectives** of this study were: to evaluate bone samples exposed to tropical climate, macro and microscopically, verifying presence of soil attached to surface, typical cells presence, matrix area and fungi quantity in order to correlate these findings with DNA amount and genetic markers amplification; and to recover DNA from these samples comparing 3 extraction methods as well as the performance of conventional STR and mini-STR markers. **Material and Methods**: Cortico-medullary fragments of femoral diaphysis were used from 20 skeletonized corpses found in Ribeirao Preto micro-region, in the period of 1998-2009. For histopathological analysis, sections were stained with H&E, methenamine silver and Periodic Acid-Schiff; and immunostained with CD31 and CD34 markers. For DNA analysis, fragments were sanded, pulverized and three extraction methodologies were used: silica columns, organic solvent and complete demineralization. After that, DNA was quantified with Quantifiler Duo Kit (Life Technologies) and genetic markers were amplified with STR and mini-STR commercial kits. **Results**: we have observed osteocytes in all samples, but in very diverse quantities (average of 0.000041 cells per μm^2). There was no expression of endothelial cell markers in all cases and fungus pronounced presence was observed in 35% of samples. By silica columns extraction method, only 5 samples indicated the amount of approximately 0.01ng/µl, on the other hand, 19 samples had values above this value by demineralization extraction protocol. Presence of reddish soil attached to bone surface could predict DNA amounts below 0.001ng/µl, with high sensitivity and specificity. Bone microstructure variables that had statistically significant correlation with lower DNA quantity and number of amplified alleles were: major loss of bone matrix area and inferior nuclei number per bone area. To obtain genetic profiles, mini-STR was much more efficient than conventional STR (50% of complete genetic profiles against 5%), proving the best performance in this sample type. **This study demonstrates** that: (1) the visual and histopathological analysis used here is an efficient tool to screen viable samples for genetic analysis; (2) selection of extraction methods capable of removing DNA bonded to mineralized matrix and the use of primers that amplify small DNA fragments can enhance molecular results. It is essential to develop strategies for improving methodologies and the laboratorial practices presented can guide the best conduct in this particular situation in human identity.

KEYWORDS: DNA; Forensic identification; Histopatological studies; Human bones.

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Dissertação de Mestrado - concorrendo a prêmio

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Dissertação de mestrado

Genetic analysis for traces of sexual crimes

Piza, PBT[1,2]; Barcelos, RSS[1,3]

[1.] Pontifícia Universidade Católica de Goiás – Mestrado em Genética, Goiânia, GO.
[2.] Instituto de Genética Forense – Secretaria da Segurança Pública do Estado do Tocantins, Palmas, TO.
[3.] Superintendencia da Polícia Técnico-Científica – Secretaria da Segurança Pública e Justiça do Estado de Goiás, Goiânia, GO.
e-mail: patriciabonilhatp@hotmail.com

In Brazil sexual violence is a crime. Rape and vulnerable rape are heinous crimes. It is estimated that only 10% of rapes are recorded and the SENASP point 42.946 police incidents in 2010. Most victims are women aged below 14 years. The absence of expert evidence difficult the sentencing of the offender. In victim, exams corpus delicti of carnal knowledge and research of semen when positive do not identify the perpetrator and negativity is not a factor of no sexual assault. Genetic analysis by PCR-STR and Y identifies the presence of DNA and the genetic profile of the perpetrator. This paper aims to analyze samples for traces of sex crimes to obtain molecular profiles of Y-STR. We selected 19 cases of sex crimes with no suspect, totaling 20 fe victims aged 11 months to 81 years, which resulted in 48 samples questioned, of which 44 were subjected to differential extraction and 4 to organic extraction, totaling 92 products extraction (44 SF, 44 NSF and 4 Organic). The DNA quantitation by real-time PCR detected the presence of DNA in 62% of the samples. Of these, 21 samples were selected and standardized to a concentration of Y-DNA 0.1 ng to 1.25 ng / PCR reaction for Y-STR. Amplification was performed for 17 joint multiplex Y-STR markers and electrophoresis capillary was preceded in genetic analyzer and the results were analyzed by programs. Of the 21 samples amplified, 12 had results for Y-STR and their haplotype been classified as full (Y-STR 17), minimum (11 Y-STR) and incomplete (absence of one or more of the Y-STR minimum haplotype) resulting 10 minimal haplotypes and complete and 02 incomplete. After comparing the minimum haplotypes and complete intra case and between their criminal cases, it was evident that the sexual assaults were committed in each case, by a single assailant, not featuring serial crimes. It is easy to recognize the importance that the Y-STR haplotype analysis assumes the sexual crimes as expert evidence, especially when it becomes the only genetic information of the offender being questioned samples obtained. The results presented in this study demonstrate the importance of being analyzed the largest number of polymorphic markers Y-STR haplotype to compose an informative, giving preference to multiplex sets that amplify multiple loci simultaneously, including polymorphic markers with products up to 200 base pairs.

KEYWORDS: Sexual crime; Expert evidence; Y-STR.

Dissertação de Mestrado - concorrendo a prêmio

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Forensic utility of mitochondrial haplogroups in Brazilian individuals derived from coding region SNPs

Favaro, EC[1]; Sumita, DR[1]; Whittle, MR[1]

[1.] Genomic Engenharia Molecular, São Paulo, SP.
e-mail: elaine@genomic.com.br

Mitochondrial DNA (mtDNA) is an important tool for studies of ancestry and for the resolution of cases in which the analysis of nuclear DNA is not possible. Sequencing of the hypervariable (HV) region is often employed, but SNPs in the coding region also assist in the discrimination beween individuals especially when they share the same HV1/HV2 sequences. These polymorphisms can be advantageously genotyped in a simpler manner, requiring smaller amounts of DNA. Furthermore, the inference of the mitochondrial haplogroup is directly obtained from the result, requiring no comparison between sequences. The aim of this study was to develop a simple multiplex PCR for genotyping SNPs present in the coding region for use in forensic cases or in studies of ancestry. Due to the extensive miscegenation in the Brazilian population the selection of SNPs was designed to cover the most common haplogroups that characterize the major world populations: Africans, Asians and Europeans (A, B, C, D, E, F, G, H, I, J, K, L1, L2, L3, M, N, N1, N9, R, R9, R9b, T, U, V, W, X and X2). Single-base extension (SNaPshot) was used for genotyping 27 SNPs. The multiplex reaction was tested in 286 individuals from the states of São Paulo and Rio de Janeiro, confirming the presence of the majority of this population haplogroup. The haplotypes identified show good correlations with HV1/HV2 region sequencing and, in some cases, information of one method was of help to the other. These samples were separated based on the phenotypic appearance of the individuals and it was observed that 91% of those with physical characteristics of the African population had the expected haplogroups. The same was observed for 89% of individuals of the Asian population, and for 41% of individuals of Caucasian background. These mtDNA coding region SNPs were also used in a case where it was necessary to determine which family, out of 25 possible families, was related to a set of badly degraded skeletal remains in which previous autosomal STR analysis had been unsuccessful. The mtDNA SNP panel narrowed the choice down to three families, after which an autosomal SNP panel was used to relate just one family to the skeletal remains, thus reducing casework and minimizing the possibility of false inclusions.

KEYWORDS: Mitochondrial haplogroup; SNP; Indel; Parentage testing; Forensic genetics.

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Is it safe to investigate kinship (parenthood) through analysis of DNA samples from oral swab?

Silva, DSBS[1]; Alho, CS[1]

[1.] Faculdade de Biociências, PUCRS, Porto Alegre, RS.
e-mail: csalho@pucrs.br

In many cases of kinship investigation, DNA samples from oral swabs are often used. However, due to the fact that the embryo is originated from the germ cells (sperm and ova), is it safe to investigate parenthood through analysis of DNA samples from oral swab? One of the stages in pattern formation in animal embryos is the positioning of cells in different embryo layers (ectoderm, mesoderm and endoderm). Each of the three germ layers gives rise to specific tissues and organs. During early embryogenesis, the ectoderm represents the external germ layer. From the surface ectoderm the epidermis and other stratified epithelia develop, giving rise to diverse specialized structures such as hair follicles, mammary glands, salivary glands and teeth. Mesoderm arises during gastrulation when cells in the ventral region of the embryo invaginate to form an inner layer of cells beneath the ectoderm. During development of the mammals› embryo, blood cells are generated from mesodermal precursors at specific times and locations. Also, the urogenital system in mammals is formed from intermediate lateral plate mesoderm, and the gonad is formed by the coordinated development of two very different cell lineages: germ cells and the somatic gonadal mesoderm that surrounds the germ cells. Since germ cells (sperm and ova) are originated from the mesoderm, primordial mutations in this embryo layer would remain in semen and blood, but not in saliva (oral cells). Mutations are rare events, but autosomal short tandem repeats (STR) used in paternity tests may present slippage or micro deletion/insertion mutation with rates in the order of $1x10^{-3}$ in one generation [parent-child]. Even without considering the unusual cases of chimerism and/or mosaicism, in Brazil more than 50 thousand families are assessed annually for identification of paternity by analysis of autosomal loci. Therefore, it is expected that cases of mutations are being investigated and that such changes might be interfering in the conclusion of the reports. Due to the chances of occurrence of mutated cell lines, the analysis of STR markers could result in erroneous conclusions. In conclusion, it is reasonable to suggest that the analysis of blood samples could reduce the chances of an error in the investigation of biological linkage, since blood and semen present the same embryonic origin, unlike the saliva (oral cells) that presents ectoderm origin.

KEYWORDS: Paternity test; Chimeris; Mosaicism.

Development and use of a single-tube 8plex PCR XSTR loci amplification assay for human identification and relationship testing

Silva, FF[1]; Francischini, CW[1]; Silveira, D[1]; Lima, MD[1]; Sumita, DR[1]; Whittle, MR[1]

[1.] Genomic Engenharia Molecular, São Paulo, SP.
e-mail: fabiana@genomic.com.br

Short tandem repeat (STR) loci are currently the markers of choice for routine casework involving human identification and human relationship testing. These markers are sufficiently polymorphic so as to have a desirable power of discrimination when used together. Commonly these loci are amplified together by PCR and the resultant alleles are separated by electrophoresis and visualized for analysis and interpretation. Although the analysis of human short tandem repeat (STR) markers located on autosomal chromosomes is sufficient for the resolution of most cases of human identification and relationship testing, some challenging situations require the use of additional markers, such as STR markers situated on the sex chromosomes. For this reason, we developed a multiplex PCR assay for human identification and relationship testing based on the amplification of eight STR loci present on the human X chromosome (DXS8377, DXS7423, GATA17D05, DXS7424, DXS7132, DXS9898 and DXS10078, DXS10135) on four different linkage groups. The markers are amplified in the same tube in the presence of fluorescent primers labeled with the FAM and JOE dyes, and subsequently submitted to capillary electrophoresis in DNA sequencers for separation and visualization. The amplification occurs directly from a single 1 mm filter paper disk containing dried blood or saliva and for forensic analyses extracted human DNA is used. The sensitivity of the amplification is less than 1 ng per reaction. An allelic ladder comprising the most prevalent alleles of the eight loci in the Brazilian population is under construction and will be used to control for electrophoretic mobility, together with the GeneMapper software for the rapid generation of genotype tables. Currently this PCR multiplex has been most useful as a complimentary tool in relationship testing in which the alleged father is deceased and has claimant daughters which must inherit one X chromosome from their biological father.

KEYWORDS: Parentage testing; X chromosome; STR loci; Multiplex PCR; Forensic genetics.

Development and use of a single-tube 21plex PCR STR loci amplification assay for human identification and relationship testing

Sumita, DR[1]; Francischini, CW[1]; Lima, MD[1]; Silveira, D[1]; Whittle, MR[1]

[1.] Genomic Engenharia Molecular, São Paulo, SP.
e-mail: denilce@genomic.com.br

Short tandem repeat (STR) loci are currently the markers of choice for routine casework involving human identification and human relationship testing. These markers are sufficiently polymorphic so as to have a desirable power of discrimination when used together. Commonly these loci are amplified together by PCR and the resultant alleles are separated by electrophoresis and visualized for analysis and interpretation. Currently there are several different kits commercially available for the multiplex PCR amplification of these loci. Earlier kits enabled amplification of 13 autosomal STR loci but more recent products permit the co-amplification of 18 loci, and latterly, of 24 loci. This increase in STR number reflects a demand for increased power of discrimination, especially in certain types of relationship testing where close relatives may need to be distinguished. In this work we describe the development and use of single tube multiplex PCR kit in which 21 commonly used (and including CODIS) autosomal STR loci (CSF1PO, FGA, F13B, Penta D, Penta E, TH01, TPOX, VWA, D1S1656, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D10S1237, D13S317, D16S539, D18S51, D19S433, D21S11, D221045), together with amelogenin, are amplified. For relationship testing the amplification occurs directly from a single 1 mm filter paper disk containing dried blood or saliva, and the loci are amplified using primers with only four different fluorophores, and for forensic analyses extracted human DNA is used. The alleles are separated and visualized using standard capillary DNA sequencers. An allelic ladder comprising the most prevalent alleles of the 21 loci in the Brazilian population is also used to control for electrophoretic mobility, together with the GeneMapper software for the rapid generation of genotype tables. Besides amplifying more autosomal STR loci than most kits presently available on the market, we have found this kit to satisfy our requirements for all parentage testing and in forensic casework. This kit and its earlier versions have been regularly employed in the successive proficiency exercises organized by the Spanish and Portuguese-Speaking Working Group of the ISFG.

KEYWORDS: Parentage testing; CODIS; STR loci; Multiplex PCR; Human identification.

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Limitations of blood typing in forensic samples

Mota, MF[1,2]; Cândido, IM[1,3]; Bezerra, LSA[1]; Godinho, NMO[1]; Carvalho, NR[1]; Vieira, TT[1]; Barcelos, RSS[1,3]

[1.] Laboratório de Biologia e DNA Forense, Polícia Técnico-Científica, Goiânia-GO, Brasil.
[2] Doutorado em Ciências da Saúde, Universidade Federal de Goiás, Goiânia-GO, Brasil.
[3.] Mestrado em Genética, Pontifícia Universidade Católica, Goiânia-GO, Brasil.
e-mail: marianafmota@yahoo.com.br

Introduction: Blood is a very important vestige of evidence in solving crimes. When there is suspicion of the presence of this trace in crime scenes, the crime scene investigator may request testing to determine if the sample is blood and whether it is human. Additionally, tests may be requested to characterize the biological material, such as blood typing and DNA analysis. However, in forensic samples, there are various difficulties with blood typing because of the quality of these samples and the time of exposure to agents that degrade the erythrocyte surface antigens. The objective of this study was to evaluate the limitations of blood typing in criminal investigations, with the report of a case of violent death. **Material and Methods:** The case involved a violent death that occurred in a rural area of the State of Goias. The goal was to differentiate a probable suicide from a homicide. Our laboratory received traces of biological material collected at the crime scene and blood collected from the cadaver at the Institute of Forensic Medicine. The following analyses were performed: blood assay by the benzidine test and human gamma and non-gamma globulin method, determination of ABO blood group by the absorption-elution method, DNA extraction using the phenol/chloroform method, PCR amplification to detect the amelogenin sex marker and 15 STR autosomal markers, using the AmpISTR Identifiler kit (Applied Biosystems) and capillary electrophoresis in the ABI 3130 Genetic Analyzer (Applied Biosystems). **Results**: The screening tests confirmed the presence of human blood in all samples analyzed. In the blood sample collected from the corpse, blood typing showed type A. In the samples collected at the crime scene, one was type A and the others type O. In a repeat analysis of the blood sample collected from the corpse, with the same method used initially, the result was type O. The molecular analysis of all samples collected at the crime scene demonstrated the presence of DNA from a single person, , whose allelic pattern coincided with that identified in the blood sample from the corpse. **Conclusion**: The absorption-elution technique is difficult to perform and has a low sensitivity and specificity. Furthermore, errors can occur due to degradation of the samples found at crime scenes, the action of microorganisms and even the interpretation of the result, and false results can cause irreparable damage to the criminal investigation. Currently, official molecular forensic laboratories are already set up for STR marker analysis, which has a higher power of identification compared to the ABO system. Thus, it is unnecessary to do blood typing in forensic samples.

KEYWORDS: Blood typing; Forensic genetics; ABO blood group; Violent death; Blood assay.

O exame de DNA na identificação de autoria de crime de estupro em série - relato de caso

Godinho, NMO[1]; Bezerra, LSA[1]; Mota, MF[1]; Cândido, IM[1]; Carvalho, NR[1]; Fonseca, BBFC[1]; Vieira, TT[1]; Barcelos, RSS[1]

[1.] Laboratório de Biologia e DNA Forense, Instituto de Criminalística Leonardo Rodrigues da Polícia Técnico-Científica, Goiânia-GO, Brasil.
e-mail: neidegodinho@gmail.com

Introdução: O criminoso em série é um tipo de pessoa de perfil psicopatológico que comete crimes com certa frequência, geralmente seguindo um *modus operandi*. O presente trabalho relata a identificação de casos de crime de estupro em série ocorridos na cidade de Anápolis, Goiás. De acordo com a autoridade policial responsável pelas investigações, o agressor agia há mais de três anos. Ele foi acusado de estuprar 12 mulheres, sendo que, em oito delas, foi realizado o exame de DNA. Os objetivos deste trabalho foram verificar a ocorrência de coincidência entre os perfis genéticos masculinos das amostras coletadas nas vítimas com os suspeitos encaminhados e, posteriormente, constatar a coincidência entre os perfis masculinos das amostras coletadas nas vítimas, entre si. **Materiais e Métodos:** *Amostras questionadas*: secreções vaginal, vulvar, anal e sangue.*Amostras referência*: sangue e suabe oral das oito vítimas e cinco suspeitos.*Extração do DNA*: extração diferencial e orgânica, com purificação em Amicon®, e NaOH, conforme o tipo de amostra. *Quantificação*: quatro vítimas tiveram as amostras quantificadas para DNA humano total e masculino no equipamento de PCR em tempo real IQ5® da BioRad, utilizando kit Plexor® HY System.*Amplificação*: as amostras foram amplificadas para 15 marcadores STR autossômicos utilizando os kits de amplificação PowerPlex® 16 HS System e/ou AmpF*ℓ*STR® Identifiler Plus. Em quatro casos, foi utilizado também o kit Yfiler®. Os produtos de amplificação foram separados por eletroforese capilar no analisador genético ABI®3130 e os perfis genéticos analisados com auxílio do programa GeneMapper® ID V3.2. **Resultados:** Entre os anos de 2010 e 2011, foi realizado exame de DNA em quatro vítimas, sendo obtido perfil genético único de origem masculina com STR autossômico e Cromossomo Y. Em uma das vítimas, foi obtido amplificação apenas para os marcadores do cromossomo Y. Quatro suspeitos encaminhados inicialmente foram excluídos. Após análise dos perfis genéticos masculinos, percebeu-se que eram coincidentes entre si. Esta informação foi repassada para a Autoridade Policial responsável, que solicitou confronto entre os perfis masculinos de STR autossômico e cromossomo Y obtidos das vítimas. Verificou-se coincidência entre eles. Na amostra em que foi obtido apenas resultados para o Cromossomo Y, a comparação só foi possível porque houve a genotipagem desses marcadores nas demais vítimas. Posteriormente, outras vítimas e um novo suspeito foram encaminhados para análise. Os resultados obtidos mostraram que o perfil genético masculino era idêntico nas oito vítimas e coincidente com o perfil do suspeito analisado.**Conclusão:** Os primeiros resultados gerados pelo laboratório foram de suma importância para orientar nas investigações e sugerir que se tratava de crime em série cometido por mesmo autor. Ressalta-se a importância de se utilizar marcadores do cromossomo Y nas análises e a necessidade de se ter bancos de dados genéticos, como o CODIS.

KEYWORDS: Violência sexual; Banco de dados; Cromossomo Y; Crime em série; DNA.

Relato de caso - concorrendo a prêmio

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The forensic anthropology as screening for forensic genetics analyses

Francisco, RA[1]; Silveira, TCP[1]; Secchieri, JM[1]; Iwamura, ESM[2]; Guimarães, MA[1]

[1.] Centro de Medicina Legal da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo – CEMEL/FMRP-USP.
[2.] Universidade Federal de São Paulo – UNIFESP.
e-mail: raffaela@usp.br

Introduction: Currently Forensic Genetics is the main tool used in identification cases. This employs the techniques of molecular biology to assist in crimes elucidation, particularly in the determination of authorship thereof. However it is known that DNA analysis is still costly and taken, many times in centers that do not have a laboratory for this purpose, having to send their samples to be analyzed elsewhere, a result thereof may take months to be obtained. As a combined technique for human identification, we include the Forensic Anthropology, which happens to be an area of knowledge that applies the methods of physical anthropology and archeology collection and analysis of legal evidence, trying to establish the identity of a human being. The forensic anthropological examination consists of a bioanthropological draw profile of the victim, including gender, age, stature, ancestry, manual dexterity, dental characteristics, bone abnormalities, bone pathologies and individual characteristics. Objective: Reduce the number of forensic DNA analysis, since the forensic anthropological analysis provides data that allows you to direct the application of DNA testing for an individual or a specific group of individuals, it is also possible to reduce the spending of a Forensic Genetics laboratory and there to optimize results. Methods: Application of the protocol for analysis of bones from the Forensic Anthropology Laboratory (LAF) as screening to forensic DNA testing. This protocol is already used in the Faculty of Medicine of Ribeirão Preto, University of São Paulo (FMRP/USP), and was created in 2005 in a project between the University of Sheffield (UK) and the Centre of Forensic Medicine (CEMEL) Faculty of Medicine of Ribeirão Preto, University of São Paulo (FMRP/USP). Discussion and Conclusion: With the results of a bioanthropological examination is possible to reduce the number of samples to be done a DNA test, which can reduce the time and cost of such examination. We report a case of positive identification and anthropological analysis as a key tool in directing DNA analysis.

KEYWORDS: Forensic genetics; Forensic anthropology; DNA; Human identification, Crimes.

Relato de caso - concorrendo a prêmio

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DNA analysis as an integration tool in scientific investigation in a murder case at São Paulo city, Brasil

Fialho, DM[1]; Peixoto-DÁvila, AV[2]; Bittencourt, EAA[1]; Camargo, ECM[3]; Rocha, T[2]

[1.] Laboratório de DNA, Núcleo de Biologia e Bioquímica, Instituto de Criminalística /SPTC, São Paulo, Brasil.
[2.] Núcleo de Perícias em Crimes Contra Pessoa, Instituto de Criminalística /SPTC, São Paulo, Brasil.
[3.] Instituto Médico Legal, SPTC, São Paulo, Brasil.
e-mail: danya.dmf@policiacientifica.sp.gov.br

Domestic violence against women is a subject that concerns public security authorities worldwide and it has been a human society recurring practice. Only in São Paulo state, between September 2001 and December 2012, about 120 women were murdered, and government statistics estimates 70% of the cases of violence against women occurred at their own residence. Here we report a case of an homicide involving a fe victim, with perfect integration between scientific investigations areas (crime scene analysis, the autopsy examination and laboratory tests). The present case was initially notified as a suspicious death, i.e., a dead body founding. The evidences recovered at the crime scene and from the postmortem analysis suggested a murder preceded by fight and the only suspect was the victim´s husband, who denied being the murder. His chest, arms and hands presented many injuries with characteristics of those caused by fingernails scratches, a typical evidence of victim´s defense. The autopsy examination revealed the cause of death was asphyxia by strangulation. DNA laboratory of Criminalistics Institute of São Paulo State was requested to analyze stains collected at the crime scene, from the victim´s shirt and to look for foreign DNA beneath victim´s fingernails. The analysis results from the stains were positive to human blood and to the victim´s DNA profile. The autosomal DNA analysis from the beneath fingernails samples showed a large amount of biological material and provided a mixed DNA profile of the victim´s and the suspect´s cells. The Y chromosome analysis revealed a perfectly coincident haplotype with the suspect´s profile. Crime scene findings and results of DNA tests were sufficient and decisive to the formal accusation of the suspect and also husband´s victim in homicide crime.

KEYWORDS: Domestic violence; Homicide; Subungueal; Crime scene.

Relato de caso - concorrendo a prêmio

Detection of tri-allelic at the TPOX locus in the case of human identification

Souza, CA[1]; Oliveira, TC[2]; Santos, SM[1,2]

[1.] Laboratório de Perícia e Pesquisa em Genética Forense, SDS, PE.
[2.] Laboratório de Imunopatologia Keizo Asami, UFPE, Recife, PE.
e-mail: carlos.souza@sds.pe.gov.br

Tri-allelic cases are observed in the TPOX locus, which often refers to a chromosomal rearrangement. There is a high possibility of the third allele is only a repeated sequence of DNA inserted near the TPOX locus on chromosome 2 or even inserted elsewhere in the genome, as in chromosome X. The tri-allelic pattern is divided into two different categories. The first relates to mutations present in only some cells and somatic three alleles show signs of unequal intensity (Type 1). The second is considered to be present in all cells and derived from a chromosomal rearrangement to produce the same signal intensity (Type 2). The objective of this paper is to describe a tri-allelic observed in the case of human identification in the state of Pernambuco. This case relates to the identification of a young man through the analysis of a fragment of muscle and blood sent to the Laboratório de Perícia e Pesquisa em Genética Forense of the Secretaria de Defesa Social de Pernambuco. The reference samples (buccal mucosa) and fragments of the sample questioned (muscle and blood) were subjected to DNA extraction using the kit DNA IQ System (Promega Corporation), according to protocols recommended by the manufacturers. The extracted DNA samples were subjected to amplification by Polymerase Chain Reaction (PCR) with the use of system analysis STRs loci "Identifiler® Plus" (Life Technology) totaling sixteen (16) loci. The amplification products the samples were subjected to capillary electrophoresis on ABI PRISM 3500 instrument HID Genetic Analyzer (Applied Biosystems) at which 15 regions (loci) STR were analyzed, and amelogenin for identifying the gender. The study of nuclear DNA polymorphisms allowed to determine the genetic profile of the sample questioned body of unknown identity confirming belonging to a person. The electropherogram analysis showed the existence of tri-allelic the TPOX locus, which, in literature, has an occurrence of a man every two women. The examination indicated the presence of tri-allelic the two types of tissue being examined which indicates a standard tri-allelic type 2. Confirmation of this possibility comes from the fact alleles 8, 9 and 10 present in the electropherogram have a balanced signal.

KEYWORDS: Forensic genetics; Tri-Allelic standard; Thyroid peroxidase; Chromosome 2; DNA.

Relato de caso - concorrendo a prêmio

Frequência genética de 17 marcadores STR numa amostra de alunos da Escola Paulista de Medicina da Universidade Federal de São Paulo

Oliveira, MA[1]; Oliveira e Sousa, MLAP[1,2]; Iwamura, ESM[1]

[1] Departamento de Patologia – Escola Paulista de Medicina-Universidade Federal de São Paulo, EPM/UNIFESP - São Paulo, SP, Brasil.
[2] Instituto de Criminalística de São Paulo – Laboratório de DNA, Núcleo de Biologia e Bioquímica, Instituto de Criminalística/SPTC, São Paulo, SP, Brasil.
e-mail: marcomavalo@gmail.com

Introdução. No Brasil, por causa da diversidade da população, há vários dados de frequência de STR destinados à análise forense de diferentes subpopulações. No entanto, a necessidade da introdução de novos marcadores STR mais sensíveis foi demonstrada em muitas situações. Material e Métodos: Neste estudo, após consentimento informado, amostras de sangue foram coletadas em papel filtro FTA de 400 voluntários independentes, com idade entre 18-26 anos , estudantes do sexo masculino e feminino da Escola de Medicina e Biomedicina, EPM / UNIFESP. Após a extração de DNA, realizou-se a PCR pelo sistema Powerplex ESX17 ® e a genotipagem compreendendo os 17 loci de STR, incluindos os midi, mini e mega. Isso permitiu que a co-amplificação, detecção de fluorescência de 17 loci (D18S51, D21S11, TH01, D3S1358, amelogenina, D16S539, D2S1338, D1S1656, D10S1248, FGA, D8S1179, vWA, D22S1045, SE33, D19S433, D12S391 e D2S441). O sequenciamento dos fragmentos foi realizado com o sequenciador ABI 3100. Resultados e Discussão: Os resultados preliminares de amostras de 356 estudantes da EPM / UNIFESP são apresentados. As 44 amostras restantes de DNA foram extraídas e amplificadas para STR 17. A PCR e o sequenciamento serão repetidos para confirmar a presença de alelos raros ou algumas discrepâncias nos resultados. Esses dados serão usados por laboratórios públicos ou privados para cálculos estatísticos complexos e para processos civis ou criminais, com a finalidade de investigar o parentesco genético ou identidade de DNA em nosso país. Além disso, este estudo é de extrema importância em casos de amostras degradadas, como ossos e especialmente para casos de DNA fragmentado, como nos tecidos fixados em formalina e embebido em parafina e amostras vestigiais. Deverão ser utilizados em laboratórios de biologia molecular em São Paulo , outros Estados do Brasil ou em outros países onde os imigrantes brasileiros estão vivendo.

PALAVRAS-CHAVE: STR, Maradores; DNA.

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Dissertação de mestrado

Allele frequencies, forensic parameters and ethnic composition of the Rio de Janeiro population, based on STR markers

Hessab, T[1,2]; Souza, M[1]; Carvalho, RM[1]; Martha, SFP[1]; Garrido, RG[1]; Freitas, NF[1]; Sallenave-Sales, SL[1]; Moura-Neto, RS[3,4]; Cabello, PH[2]

[1.] Instituto de Pesquisa e Perícias em Genética Forense, DGPTC/PCERJ, Rio de Janeiro, RJ.
[2.] Instituto Oswaldo Cruz, Fiocruz, Rio de Janeiro, RJ.
[3.] Instituto de Biologia, UFRJ, Rio de Janeiro, RJ.
[4.] DIMAV, Inmetro, Rio de Janeiro, RJ.
e-mail: hessab@gmail.com

In human genetic identification, after comparing genetic profiles, statistical methods are applied to estimate the probability that the DNA in a random population sample has the same profile as the DNA evidence sample. This analysis is based on frequencies obtained from previous studies done in the population. In the state of Rio de Janeiro, the IPPGF (*Instituto de Pesquisa e Perícias em Genética Forense*) is responsible for performing genetic identification in criminal cases. This Institute receives samples of the whole state, joining a representative stock of Rio de Janeiro samples. Therefore, this work aims to analyze the DNA profiles of 505 unrelated individuals who, from 2008 to 2011, donated biological material to IPPGF. For this purpose, we have genotyped 17 autosomal STRs (short *tandem* repeats) using the human identification kits PowerPlex® 16 System (Promega Corporation) and AmpFℓSTR™ Identifiler (Applied Biosystems). Based on the genetic profiles analysis, we have calculated the STR allele frequencies, tested for Hardy-Weinberg equilibrium, inbreeding coefficient (F_{IS}), forensic parameters and performed analyses to study the ethnic composition of the population. We detected a total of 215 distinct alleles, in the 17 genetic markers. The *locus* D21S11, was the most polymorphic (25 alleles), followed by FGA (22 alleles). The TPOX allele 8 was the most frequently observed. Furthermore, this *locus* had the lowest Power of Discrimination (PD = 0.879), Power of Exclusion (PE = 0.419) e Polymorphism Information Content (PIC = 0.676) and the highest Probability of a Random Match (PM = 0.121). The marker Penta E had the highest Power of Discrimination (PD = 0,983), Power of Exclusion (PE = 0.761) and Polymorphism Information Content (PIC = 0.905) and the lowest Probability of a Random Match (PM = 0.017). Further analyses were performed to study the ethnic composition of the population. The Neighbor-Joining phylogenetic tree (data from different Brazilian regions) showed a similarity between the Rio de Janeiro population and the Northeast Brazilian population. However, none statistical significance was observed in pairwise F_{ST} analysis among populations, indicating the absence of structuration, an important trait for genetic markers used in human identification tests. The "Triangle Plot" graphic, designed in the software Structure, suggests that, on average, individuals have no greater contribution of a particular ethnic group. By comparing the allele frequencies obtained, we have detected that the alleles of higher frequency, in the present study, showed in general, wide distribution in various populations.

KEYWORDS: Forensic genetics; Population genetics; STR polymorphisms; Human identification, Rio de Janeiro.

Dissertação de Mestrado - concorrendo a prêmio

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DISSERTAÇÃO DE MESTRADO

Ancestry markers in regions of high frequency for oral clefts

Vieira Machado, CD[1,6]; Hermida, RMSM[1]; Chaves, AC[1]; Bailliet, G[2]; Poletta, FA[3,6]; Mereb, JC[4]; López-Camelo, J[2,3,6]; Castilla, EE[3,5,6]; Orioli, IM[1,6]

[1.] ECLAMC, Departamento de Genética, Instituto de Biologia, Rio de Janeiro, Brasil.
[2.] ECLAMC, Instituto Multidisciplinario de Biología Celular (IMBICE), La Plata, Argentina.
[3.] ECLAMC, Centro de Educación Médica e Investigaciones Clínicas "Norberto Quirno"(CEMIC), Buenos Aires, Argentina.
[4.] ECLAMC, Hospital de Area de El Bolsón, Río Negro, Argentina.
[5.] ECLAMC, Laboratório de Epidemiologia de Malformações Congênitas, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil [6]Intituto Nacional de Genética Médica Populacional (INAGEMP).
e-mail: camilladvm@gmail.com

Cleft lip with or without cleft palate (CL/P) affects approximately 1/700 live births with a higher frequency reported in populations with Asian and Native American ancestry. A recent study from the ECLAMC (Latin American Collaborative Study of Congenital Malformations) identified clusters with high CL/P prevalence: Argentinean Patagonia and Southern Chile, Northwest Argentina, Bolivia and a contiguous region between Ecuador and Colombia. These regions were associated with risk factors such as high altitude, low socioeconomic status and Amerindian ancestry. In this work our main objective was to investigate if there is a relationship between the degree of Amerindian ancestry and susceptibility to CL/P in the high prevalence clusters mentioned above. We compared in a case-control study the frequency distributions of the mtDNA haplogroups (A, B, C, D, and others) and of two Y chromosome markers: DYS199, characterized by a C>T transition with high frequency in Native American populations, and YAP, an Alu insertion that suggests African ancestry. To verify the independence of these frequency distributions,between cases and controls, we used the chi-square test. There was no difference in the proportion of each Amerindian mitochondrial haplogroup (A, B, C and D) between cases and controls. The conjoined frequency of Amerindian mitochondrial haplogroups was higher in cases compared to controls in Argentinean Patagonia (88,71% and 72,87%), and Ecuador/Colombia (98,44% and 85,67%); not different between cases and controls in Southern Chile (95.71% and 94.47%%), Northwest Argentina (98,73% and 92,86%), and Bolivia (97,30% and 92,05%). Similar patterns were observed for the DYS199T allele with differences between cases and controls in Argentinean Patagonia (98,57% and 18,61%), Ecuador/Colombia (58,54% and 29,10%), and in Southern Chile (25.93% and 7.41%); there was no difference between cases and controls in Northwest Argentina (11,54% and 10,71%), and Bolivia (62,5% and 72,62%). The YAP+ allele suggestive of African ancestry had different behavior in the clusters, being absent in the Southern Chile; it was less frequent in cases (4.35%) than in controls (28.00%) in the Northwest Argentina; had the same frequency in cases and controls in Argentinean Patagonia (7.69% and 10.46%) and Bolivia (20.00% and 13.04%); and was more frequent in cases (41.18%) than in controls (10.69%) in Ecuador / Colombia. The clusters showed no heterogeneity in the relative incidence of CL/P in Amerindians, which was 2.9 (1,63 – 5,25) times higher in cases than in controls using mtDNA data and 2.4 (1.65 – 3.39) times higher in cases than in controls using the DYS199 marker. The higher frequency of the YAP + allele in Ecuador/Colombia CL/P cases could be associated with parental consanguinity and/or low socioeconomic status, also observed previously in this cluster.

KEYWORDS: Oral clefts; Mitochondrial DNA; Y chromosome; Ancestry; ECLAMC.

Dissertação de Mestrado - concorrendo a prêmio

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Dissertação de Mestrado

Comparison of DNA extraction methods from human bone using magnetic bead and silica column

Cândido, IM[1,3]; Bezerra, LSA [1]; Mota, MF[1,4]; Godinho, NMO[1]; da Silva, RF[2]; Mendes, SDSC[2]; Barcelos, RSS[1,3]

[1.] Laboratório de Biologia e DNA Forense, Polícia Técnico-Científica, Goiânia-GO, Brasil.
[2.] Seção de Antropologia e Odontologia Legal, Polícia Técnico-científica, Goiânia-GO, Brasil.
[3.] Mestrado em Genética, Pontifícia Universidade Católica, Goiânia-GO, Brasil.
[4]. Doutorado em Ciências da Saúde, Universidade Federal de Goiás, Goiânia-GO, Brasil.
e-mail: imarquescandido@gmail.com

Introduction: DNA research has proven an effective tool and is thus widely used in forensic sciences. The identification of human remains in cases of decomposition, charred skeletal remains and mass disaster can be performed by forensic genetics, even when bones and teeth are the only viable source for DNA typing. In these cases, the quantity and quality of DNA extracted from bone samples are crucial to the success of genetic identification. The objective of this study was to evaluate two different techniques of DNA extraction in twenty-five human bones on the basis of DNA quantification and STR profiles obtained for each sample. **Material and Methods**: We randomly selected at the Laboratory of Biology and DNA Forensics of the State of Goiás, Brazil twenty-five (25) human identification cases where the femur was the sample in question. All bone samples received the same pre-treatment steps. Twenty-five aliquots of 100 mg powdered bone sample were extracted using two different techniques: magnetic beads with the Prepfiler BTA kit (Applied Biosystems) using the extraction platform Automate Express (Applied Biosystems) and a silica column wherein samples were processed manually with the QIAamp DNA Investigator kit (Quiagen). DNA extracts were quantified by real-time PCR and amplified by PCR using the Identifiler Plus kit (Applied Biosystems). The amplification products were analyzed by capillary electrophoresis in an ABI 3130 Genetic Analyzer (Applied Biosystems) using POP4 polymer (Applied Biosystems) and GeneMapper software (Applied Biosystems). **Results**: With the exception of three bones, the amount of DNA extracted by magnetic beads was up to four times higher than with the silica column. The number of loci amplified with DNA using magnetic beads was equal to or greater than that using the silica column, in all but one samples. We obtained ten complete genetic profiles (16 loci amplified in each one) with Prepfiler BTA, compared to only five full profiles with QIAamp DNA Investigator. The internal PCR control was not found to be higher than expected in any of the samples extracted by the two methods. The analysis time of the extraction procedure with magnetic beads was shorter because the incubation with lysis buffer was only 2 hours. **Conclusion**: Automated DNA extraction using magnetic beads was shown to be an excellent choice for routine genetic identification of human bone in a forensic laboratory.

KEYWORDS: Forensic genetics; DNA extraction; Bone; STR; Automation.

Dissertação de Mestrado - concorrendo a prêmio

Allele frequencies and population data for 17 Y-STR loci in Paraíba population, Brazil

Rêgo, GEQ[1,2]; Monte, SMC[2,3]; Moura-Neto, RS[4]; Oliveira, NFP[1]

[1.] Departamento de Biologia Molecular, Centro de Ciências Exatas e da Natureza, Universidade Federal da Paraíba, Cidade Universitária – Campus I- João Pessoa-PB, 58059-90, Brazil.
[2.] Laboratório de DNA, Instituto de Polícia Científica da Paraíba, R. Antônio Teotônio, s/ nº, Cristo Redentor, João Pessoa-PB, 58071-620 Brazil;
[3.] Laboratorio de Identificación Genética de la Universidad de Granada, Avda. de Madrid 11 18071 Granada, España
[4.] Instituto de Biologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-902, Brazil and DIPRO, Instituto Nacional de Metrologia Qualidade e Tecnologia, Duque de Caxias, RJ 25250-020, Brazil.

e-mail: germanarego@ig.com.br

Paraíba is one of the states in northeastern Brazil and its population represents a typical sample of Brazilian mixed ethnicity, composed mainly of European, African and Amerindian descendents. A convenience sample of 300 unrelated subjects >18 years old, male, were recruited for the study. Samples of epithelial oral cells were collected by swab. Genomic DNA was purified using ChargeSwitch® Forensic DNA Purification (Invitrogen). The haplotype DYS456, DYS389I, DYS390, DYS389II, DYS458, DYS19, DYS385a, DYS385b, DYS393, DYS391, DYS439, DYS635, DYS392, GATA H4, DYS437, DYS438 and DYS448 were typed with the commercial kit AmpFℓSTR® Yfiler™ (Life Technologies). PCR amplification was performed in a GeneAmp® PCR System 9700 (Life Technologies). Amplicons were subjected to capillary electrophoresis and typed in an ABI PRISM 3130 Genetic Analyzer (Life Technologies). Analysis of 17 Y-STRs was performed using the GeneMapper v3.2 software (Life Technologies). Allele designations were based on comparison with the allelic ladder provided in the commercial kit by the manufacturer. Allele and haplotype frequencies were estimated by simple-count method. Haplotypes and gene diversities were estimated according to the equation described by Nei. Genetic distance (Slatkin's Rst) values were assessed for statistical significance level of 5%, assuming the stepwise mutation model, as implemented in the ARLEQUIN software ver. 3.5. It was detected a total of 276 different 17-*loci* haplotypes among the 300 unrelated individuals investigated, 254 of which were unique. It was detected 20 haplotypes that appeared twice and 2 were observed three times in the investigated population. The Paraiba population showed a genetic diversity in 17 *loci* ranging from 0.4648 (DYS458) to 0.8644 (DYS385). The haplotype diversity for all 17 *loci* was 0.9994. The set of 17 Y-STR *loci*, an individual discrimination power of 0.9134 was estimated. The comparative study conducted on genetic distance between Paraíba, Portugal, Angola, Native Americans and regions of Brazil showed no statistically significant genetic differences in relation to Portugal and Brazilian regions, except the Central west region. This is the first report of Y-STR data used for genetic identification purposes in the Paraiba population, Brazil, and our results showed that verified statistical parameters are useful for forensic applications. This study was supported by DNA Laboratory of Paraiba´s Police, Brazil.

KEY-WORDS: Y-chromosome; STR; Brazil; Population data.

Amplificação direta de suabes em amostras referência utilizando o Kit de amplificação PowerPlex16HS®

Lott, KPS[1,2], Bastos, GV[1], Castro, SG[1,4], Gambarra, CLA[1], Giustina, ACB[1], Lucena, SM[1], Rêgo, GEQ[1,4], Monte, SMC [1,3]

[1.] Gerência Operacional de Análises em DNA, Instituto de Polícia Científica, João Pessoa, PB
[2.] Especialização em Biologia Molecular, Universidade Estadual de Pernambuco, Recife, PE
[3.] Doctorado en Genética e Evolución de la Universidad de Granada, España
[.4.] Mestrado em Biologia Molecular, Universidade Federal da Paraíba, João Pessoa, PB

e-mail: karinepequeno@ibest.com.br

O isolamento e purificação do DNA de amostras forenses envolvem diversas etapas que incluem processos de centrifugação, períodos de incubação, uso de detergentes, enzimas, membranas filtrantes ou resinas magnéticas em um processo bastante demorado e de alto custo. A capacidade de amplificar o DNA diretamente de suabes bucais permite que o processamento de amostras de uso corrente na rotina laboratorial seja muito mais rápido, econômico e eficiente. A amplificação direta elimina a necessidade de extração de moléculas de DNA, diminuindo os custos, os riscos de contaminação e os erros de procedimento pela redução do número de etapas, além de acelerar o tempo para o resultado final. Artigos científicos já foram publicados testando a amplificação direta de amostras preservadas em cartão FTA® e cartão 903®, contudo não foi encontrado na literatura nenhum relato de amplificação direta em amostras bucais coletadas com suabes. Nesta pesquisa foram testadas amostras de suabe bucal, método não invasivo e indolor e que está padronizado no laboratório de DNA do Instituto de Polícia Científica da Paraíba para coleta de amostras referência. Foram testadas alíquotas de 4mm^2 de 46 amostras de suabe bucal, em duplicata, adicionadas diretamente a um volume final de 25 µl e de 12,5 µl do Sistema PowerPlex® 16HS (Promega Corporation) para amplificar as regiões STR do DNA. Utilizou-se para a análise do DNA amplificado o sequenciador genético ABI Prism 3130 (Life Technologies) e os dados foram interpretados pelo software GeneMapper ID v3.2. Todas as amostras de DNA testadas utilizando a amplificação direta com volume final de 25 µl apresentaram perfis genéticos completos, com tamanho dos picos variando entre 1000 e 8000 RFU´s. Entretanto, as reações nas quais foram utilizados volumes de 12,5 µl resultaram em 70% de perfis completos, 26% de perfis parciais e 4% não geraram perfis analisáveis. Estes resultados sugerem que é possível realizar a amplificação direta em amostras bucais coletadas com suabe, com melhor desempenho em reações com volume final de 25 µl. Testes de amplificação direta em amostras de suabe bucal nos sistemas de amplificação Identifiler® Plus (Life Technologies) e NGM® (Life Technologies) também estão sendo realizados por esse laboratório.

DESCRITORES: Extração de DNA; Amplificação direta; Suabe.

Forensic applicability and association of haplotypes from the SLC45A2 gene with human pigmentation traits in Brazil

Fracasso, NCA[1]; Andrade, ES[1]; Andrade, CCF[1]; Zanão, LR[2]; Silva, MS[2]; Marano, LA[1]; Wiezel, CEV[1]; Donadi, EA[3]; Simões, AL[1]; Mendes-Junior, CT[2]

[1.] Departamento de Genética, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14049-900, Ribeirão Preto-SP, Brazil;
[2.] Departamento de Química, Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, 14040-901, Ribeirão Preto-SP, Brazil;
[3.] Divisão de Imunologia Clínica, Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, 14048-900, Ribeirão Preto-SP, Brazil.

e-mail: nadiadeaguiar@usp.br

The SLC45A2 gene encodes the Membrane-Associated Transporter Protein (MATP), which mediates melanin synthesis by tyrosinase trafficking and/or proton transportation to melanosomes. Two coding SNPs (E272K and L374F) have been associated with variation in human pigmentation. Yuasa et al. (Ann Hum Genet 2006:802-811) studied a set of 12 SNPs and observed different haplotype distributions in Germans, Japaneses and Sub-Saharan Africans. Notwithstanding the SLC45A2 diversity and its potential informativeness, the correlation of these haplotypes with human pigmentation traits was not evaluated in the Brazilian population. Since the determination of eye, hair and skin pigmentation of unknown samples found in crime scenes would be of great value for forensic caseworks, the present study aimed at evaluating the influence of SLC45A2 haplotypes in the determination of such pigmentation traits in a highly admixed population sample. To achieve this goal, 12 SLC45A2 SNPs (Yuasa et al., 2006) were evaluated in 150 unrelated individuals from the Ribeirão Preto area, located at the Northwestern region of the São Paulo State, Brazil. Individuals were classified in different groups according to the presence or absence of freckles and eye (blue, green, brown and black), hair (red, blond, brown and black) and skin pigmentation [six levels (I to VI), according to the Fitzpatrick (1988) classification scheme]. DNA was extracted by a salting-out procedure and SNPs were genotyped either by PCR-RFLP or Allele-Specific PCR, followed by Polyacrylamide Gel Electrophoresis (PAGE) colored by silver staining. Haplotypes, designated according to Yuasa et al. (2006), were determined by the following SNPs: rs732740, rs250413, rs181832, rs3776549, rs3756462, rs26722 (E272K), rs2287949 (T329T), rs250417, rs16891982 (L374F), rs40132, rs35394, and rs3733808 (V507L). Haplotypes of each individual were inferred by two independent computational methods: PHASE and PL-EM. These methods presented same results for 139 (92.7%) individuals, with average probabilities of 0.9589 and 0.9836, respectively. Haplotype frequencies were estimated using the direct counting method and were compared between groups. Two-sided Fisher's exact test, odds ratios (OR), and 95% confidence intervals (95%CI) were calculated by using the GraphPad InStat 3.05 software. Considering the 139 individuals, 23 different (10 new) haplotypes were identified. Seven haplotypes presented at least one association with a pigmentation feature, and skin color was influenced by five of them. Haplotype hp9, for instance, was associated with the presence of blond/red hair, pale skin and freckles, and also with absence of dark eyes, hair and skin. All haplotypes significantly associated with dark or light pigmentation features harbor the 374L and 374F alleles, respectively. However, it is noteworthy that the remaining set of haplotypes harboring the 374F allele is more frequent among individuals with intermediate than light pigmentation. These results reinforce the relevance of SNP L374F in human pigmentation, but also suggest an important role played by its surrounding variation.

KEYWORDS: SLC45A2; SNPs; Human pigmentation.