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Short communication

Fourteen short tandem repeat loci Y chromosome haplotypes: Genetic analysis in populations from northern Brazil

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ABSTRACT

Fourteen Y-STR loci (DYS458, DYS439, Y-GATA H4, DYS576, DYS447, DYS460, DYS456, YGATA A10, DYS437, DYS449, DYS570, DYS635 or Y-GATA C4, DYS448 and DYS438) were analysed in 873 males from eight northern Brazil populations: Belém ($N = 400$), Santarém ($N = 69$), Manaus ($N = 75$), Macapá ($N = 65$), Palmas ($N = 30$), Rio Branco ($N = 32$), Porto Velho ($N = 135$) and Boa Vista ($N = 67$). A total of 871 different haplotypes were identified, of which 869 were unique. The panel's estimated total haplotype diversity (HD) is 0.9988, and its discrimination capacity (DC) is 0.9980. The lowest estimates of genetic diversity correspond to markers Y-GATA H4 (0.550) and DYS460 (0.581), and the greatest (above 0.700) to markers DYS458, DYS576, DYS447, YS449, DYS570 and DYS635. The genetic parameters obtained were higher for the 14-Y-STR panel than that for the minimum haplotype set (HD = 0.9969; DC = 0.76) and the parameters were similar to those obtained with the panel of 17 YSTR of YHRD (HD = 0.9987; DC = 0. 9870). The analysis of molecular variance (AMOVA) indicated that most of the genetic variance is found within populations and a smaller, but significant part, is found among populations ($R_{ST} = 0.027$, p value = 0.009). The data when compared with those from African, Amerindian and European populations have shown no significant genetic distance between northern Brazil populations and Europeans, but there is a significant genetic distance when compared to Africans and Amerindians. The discrimination capacity of the markers shows a high potential for forensic analysis.

1. Introduction

Short tandem repeats (STRs) have high individual discriminatory power and have been the most widely used markers in forensic analysis. Although autosomal STRs are the most widely employed in these kinds of analyses, Y-STRs are also employed as valuable tools in human identification studies and provide more specific information, such as: (i) identification of the male component in cases of sexual crime against women; (ii) determination of the number of male donors in cases of sexual assault with two or more criminals; (iii) identification of missing people in mass disasters by comparison with paternal relatives of same lineages; and (iv) determination of paternity, especially in the absence of the alleged father [1].

Due to the relatively low discriminatory power of the minimum haplotype, many sets of Y-STR markers have been used in forensics and population analysis in different laboratories [2–5], all with the goal of achieving greater discriminatory power between haplotypes of the Y-DNA.

In this study, we developed and validated a panel of 14 Y-STR (DYS458, DYS439, Y-GATA H4, DYS576, DYS447, DYS460, DYS456, Y-GATA A10, DYS437, DYS449, DYS570, DYS635 (or Y-GATA C4), DYS448 and DYS438) that can be genotyped in a single multiplex PCR reaction, with subsequent separation and allele identification by capillary electrophoresis. The panel was validated in a sample of 873 individuals from eight populations of northern Brazil characterised by an intense admixture process between Europeans, Africans and Native Americans. We also analysed the same sample of individuals for the nine markers that make up the minimum haplotype. The efficiency of the developed panel to solve forensic cases was assessed by comparing forensic statistical parameters obtained from these markers with those estimated from the markers that make up the minimum haplotype and the panel of 17 markers of the Y-Chromosome Reference Database-YHRD [6].

2. Materials and methods

2.1. Investigated populations and DNA extraction

This study was conducted in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Blood samples

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Fig. 1. Geographic location of the cities from northern Brazil.

and swabs containing oral mucosa were collected after informed consent from 873 healthy and paternally unrelated adult subjects from eight populations from cities located in northern Brazil: Belém ($N = 400$) and Santarém ($N = 69$), both in Pará state; Manaus, in Amazonas state ($N = 75$); Macapá, in Amapá state ($N = 65$); Palmas, in Tocantins state ($N = 30$); Rio Branco, in Acre state ($N = 32$); Porto Velho, in Rondônia state ($N = 135$); and Boa Vista, in Roraima state ($N = 67$). The geographic distribution of the studied populations is shown in Fig. 1.

DNA was extracted from biological samples using the conventional organic extraction protocol with phenol-chloroform [7]. The samples were quantified in a NanoDrop ND-1000 (NanoDrop Technologies, USA).

2.2. Primers design

Fourteen markers were chosen based on the high genetic diversity described in previous work published in different continental populations [2,3,8]. The primers were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi). The possibility of these primers mispriming was tested with BLAT (http://genome.ucsc.edu/cgi-bin/hgBlat). The possibility of forming secondary structures or dimers between the primers was tested using Autodimer software [9].

General information about the fourteen Y-STR markers investigated here, such as the sequences of each primer, the GenBank access code, the position of each marker on the Y chromosome, the fluorescent label employed on the panel, variation in size amplicon, the final concentration of each primer used in PCR and the alleles amplified with the control DNA 007 provided in the commercial kit Y-Filer (Applied Biosystems), are presented in Table 1.

2.3. Cycling conditions

The PCR with simultaneous amplification of 14 markers was standardised to a final volume of 10 μL using QIAGEN® Multiplex PCR Kit (Qiagen, Hilden, Germany). The reactions were optimised to amplify genomic DNA amounts between 0.5 ng and 10 ng. Amplification reactions were performed in a GeneAmp 9700 PCR Systems thermocycler (Applied Biosystems). The cycling conditions were as follows: initial denaturation at 95 °C for 15 min, followed by 10 cycles at 94 °C for 30 s, 60 °C for 90 s, 72 °C for 60 s, 20 cycles at 94 °C for 30 s, 58 °C for 90 s, 72 °C for 60 s and finally, a final extension at 72 °C for 60 min.

2.4. PCR product detection, designation of alleles and sequencing reaction

One microliter of the amplified product was mixed with 8.5 μL of Hi-Di deionised formamide and 0.5 μL of GS-500 ROX (standard molecular weight) and analysed on the ABI 3130 Genetic Analyzer (Applied Biosystems).

Fragment size determination and allele designation was made with GeneMapper v3.2 (Applied Biosystems). The average sizes of alleles and sizes of the window detection of the alleles (bin) were initially determined by analysing at least 100 different samples. After the initial genotyping, DNA samples from the most frequent alleles were sequenced to identify the correct number of repeats. As quality control for allelic reading of the markers DYS458,