A importância da genotipagem da região codificante do DNA mitocondrial para a utilização forense: o caso do SNP 3010

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RESUMO

As análises forenses do DNA mitocondrial humano (DNAmt) são normalmente realizadas através da análise das regiões hipervariáveis por técnica de sequenciamento, mas a informação da região codificante pode ser útil para distinguir perfis idênticos, cooperando para uma identificação incontestável. No presente estudo, o SNP 3010 da região codificante do DNAmt foi avaliado usando PCR em tempo real em uma população miscigenada brasileira com perfis idênticos. Foram analisadas amostras de sangue de 160 indivíduos residentes na região metropolitana de São Paulo, Brasil. O SNP 3010 foi suficientemente discriminatório para distinguir os indivíduos que compartilhavam o mesmo haplótipo em dois dos treze casos. Além disso, o SNP 3010 é altamente discriminatório para indivíduos classificados como o haplogrupo H europeu e compartilhando o mesmo perfil. Foi possível concluir que, como os SNPs de regiões codificadoras do DNAmt não estão presentes nos bancos de dados atuais, futuros esforços são necessários para a criação de bancos de dados com dados populacionais de genomas mitocondriais inteiros, para que os mesmos possam ser utilizados em análises forenses.

Palavras-chaves: Polimorfismo, discriminatório, haplogrupo, Brasileiro

The importance of mitochondrial DNA coding region snp genotyping for forensic purpose: the SNP 3010 case

ABSTRACT

Human forensic mitocondrial DNA (mtDNA) analyses are typically performed by sequencing fragments of hypervariable regions, but coding region information can be useful for distinguish identical profiles, cooperating to an undoubtedly identification. In the present study, the mtDNA coding region SNP 3010 was examined using Real Time PCR in an admixtured sample population from Brazil with identical profiles. Blood samples from 160 individuals who are residents in the metropolitan area of São Paulo city, Brazil, were analyzed. The SNP 3010 was sufficiently discriminatory to distinguish individuals who shared the same haplotype in two of thirteen cases. Moreover SNP 3010 is highly discriminatory for individuals classified as the European haplogroup H and sharing the same profile. It was possible to concluded that, as coding region SNPs are not present in actual mtDNA databases, future databasing efforts are needed for the development of entire mtDNA genome reference population data suitable for forensic comparisons.

Key words: Polymorphism, discriminatory, haplogroup, Brazilian

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INTRODUCTION

DNA analysis for human identification uses the genetic profile of an individual based on the combination of diverse markers that are inherited of its ancestors. These markers are generally differences in sequences of nuclear DNA between individuals (polymorphisms). In some cases, however, analysis of the nuclear DNA cannot be applied. It occurs when the DNA sample is degraded or in cases which biological material does not content nuclear DNA (i.e., hair fragments). In these cases, the mitochondrial DNA (mtDNA) analysis is the choice method (ANJOS et al., 2004). The human mt genome consists of 16,568 nucleotides and is presented as a circular double-stranded (ANDERSON et al., 1981). Most of the genome comprises genes. However, a noncoding region of approximately 1,200 nucleotides, flanking the position "0" of the genome, is known as control region (CR), D-loop or hypervariable region (HV). The mtDNA hypervariable region is interesting for human identification because of its high degree of sequence polymorphisms, and comprises three segments: HV1 (position 16024-16365), HV2 (position 73-340) and HV3 (position 438-576) (LUTZ et al., 2000) . The first two (HV1 and 2) are used in forensic routine, the third region (HV3), however, has been explored in recent years (HOONG & LEK, 2005 ; ZANG et al., 2005; LEE et al., 2006), besides being important for the correct classification of some samples in their respective haplogroups (PARSON & BANDELT, 2007). Analysis of the entire mtDNA control region, including HV3, is also being proposed, for the best population haplogroup affiliation (PANETO et al., 2010). Since some sequences from HV1, HV2 and HV3 analysis have been reported as very common in different populations (same haplogypes), it is difficult to distinguish between some individuals from same mt origin. Beside control region analysis, there is high potential existence of additional variation located outside this region that can be used to discriminate against those individuals who show the most common haplotypes. These situations can be resolved with the use of Single Nucleotide Polymorphisms (SNPs) mapped out the mtDNA control region and, therefore, localized in the coding region. This approach is recent and some groups have already demonstrated the potential value of discrimination when used panels of coding region SNPs in different populations (FRIDMAN et al., 2011).

The sequencing performed by forensic laboratories usually targets HV1 and HV2, as these, approximately, 600 base pairs provide the single greatest opportunity within the mt Genome for inter-individual differentiation. When sufficient discrimination is not obtained by HV1 and HV2 sequencing, additional portions of coding region may be sequenced. It is important to say that haplogroup affiliation is not possible using only sequencing results of control region, nevertheless, it is sometimes done, but those SNPs complement the correct affiliation on subhaplogroups (PANETO et al., 2011). The presence of common mtDNA haplotypes results in situations for which even entire CR sequencing does not provide enough variation for sample discrimination.

Brazilians form one of the most heterogeneous populations in the world, result from five centuries of interethnic crosses among peoples from three continents: Europe, represented by Portuguese, Italian, Spanish, Dutch, Japanese and others; Africa, represented by African slaves; and America, the autochthonous Amerindians. In Brazil, almost all population shows some degree of admixture and the extent of admixture varied, depending on the country region analyzed (ALVES-SILVA et al., 2000). In the present article, we follow this approach by genotyping the coding region SNP 3010 to better distinguish individuals who share the same haplotype in noncoding region in order to a future application in forensic casework.

MATERIAL AND METHODS

Sample Collection and DNA Extraction

Blood samples from 160 individuals, randomly selected from the population, who are residents in the metropolitan area of São

Paulo city, Brazil, were analyzed. All the participants gave their written informed consent prior to their inclusion in this study, which was approved by USP/São Paulo Ethical Committee (FCF/USP, number 32).We used blood spotted on filter paper and DNA was extracted from two discs of 1.2 mm using DNA IQ System (Promega).

Real Time PCR Amplification and Detection

TaqMan Allelic Discrimination assays for detection of SNP 3010 were designed using by Life Technologies by Applied Biosystems (Assay by design). The primers and probes used to amplify the regions described above are listed in Table 1. These primers were designed to obtain PCR fragments that are short enough for easy amplification (ADACHI et al., 2004). A working master mix was prepared that contained 1.25 μL of SNP Genotyping Assay Mix (20X), 12.5 μL TaqMan Universal PCR Master Mix, 4 ng of human genomic DNA and purified water sufficient to 25 μL. Plates were loaded into Applied Biosystems 7500 Real-Time PCR System and AB 7500 v 2.0.5 software was used to run the genotyping assay experiment following the default standard Allelic Discrimination genotyping assay protocol. The initial step of this protocol includes pre-reading of the plate where the background florescence is recorded followed by AB standard PCR protocol of 95°C for 10 min, 95°C for 15 sec and 60°C for 1 min, repeating steps 2-3 for 40 cycles. The post-read step follows after completion of the PCR step where a post-read is performed. The software analyzed before and after florescence level and calculated the normalized dye fluorescence (ΔRn) as a function of cycle number for Allele1 (wild-type) or Allele2 (mutant).

Control Region Analysis and Haplogroup Affiliation

The entire mtDNA CR was PCR amplified using primers L15997 and R639 and sequenced using BigDye Terminator Cycle Sequencing Reaction Kit v3.1 (Life Technologies) as described by Parson & Bandelt (2007). Electrophoresis was performed on 3500 and 35009l Genetic Analyzer (Life Technologies, Foster City, CA, USA). Sequences were assigned to mtDNA haplogroups based on particular polymorphisms of each mtDNA lineages using mtDNAmanager and Phylotree (MTDNAMANAGER, 2007; VAN OVEN & KAYSER, 2008).

RESULTS AND DISCUSSION

Within a total of 160 samples analyzed, 143 (89.37%) had the G allele (wild-type) and 17 (10.63%) had the allele A (mutant). The data in table 2 show the statistical outcome of the SNP 3010 in this study and in other populations described in the Human Mitochondrial Genome Database (mtDB) (INGMAN & GYLLENSTEN, 2006). Control Region haplotypes and haplogroups affiliations are presented as supplementary material (Table S1).

It was observed that this SNP presents more polymorphic in Asian, European and Native American populations. The Brazilian population, as an example of a mixed population, showed a distribution below the total average. However, this SNP was sufficiently discriminatory to distinguish individuals with the same HV haplotype in two of thirteen groups with more than one individual with the same haplotype (about 15%). In both cases, individuals were classified as the European haplogroup H. When we classify our samples into haplogroups (African, Native American and Europeans), the distribution of alleles was clearly altered, with the allele "A" in this 4.9% of Native Americans (present only in haplogroup D), 4.0% of Africans (present only in haplogroups L2 and L4) and 27.3% of Europeans (present only in haplogroups H, J, U) (Figure 1).

Coble et al. (2004) studying 241 American individuals (classified as European haplogroups) concluded that SNP 3010 is extremely useful in forensic discrimination, particularly in haplogroup H, and also is a good target because it´s a well characterized polymorphism, reported widely in the literature, and not suspected to be associated

with disease or other manifestation. However, Billing‑Ross et al. (2016) showed that analysis of mitochondrial genomes in Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) cases indicates that individuals of a certain haplogroup or carrying specific SNPs are more likely to exhibit certain neurological, inflammatory, and/or gastrointestinal symptoms. Patients with the allele "A" of SNP 3010 suffered from higher frequency of and distress symptoms related to sleep disorder.

Bilal et al. (2008) correlated the SNP3010 with longevity in Japaneses. However, since it is a mutational hotspot found in many disparate haplogroup it is difficult to convincingly demonstrate and/or validate any possible association of these hotspot polymorphisms with longevity. Olçen et al. (2011) studied 9 mitochondrial SNP sites, including position 3010, in a Turk population. Of 30 individuals, 5 individuals showed the mutant allele 'A' (6%). Köhnemann & Pfeiffer (2011) showed that mtDNA analysis of SNPs, including SNP 3010, can be extremely helpful in cases when many hairs have to be investigated, as a fast and economic screening method. mtDNA SNP analysis is also the method of choice for challenging and old DNA samples which can be found in bones and teeth. Cases like Titanic´s shipwreck (1912) also used coding region SNPs to identify unknown bodies, incluing the position 3010. The example demonstrates the benefit of targeted mtDNA coding region typing in difficult forensic cases and highlights the need for entire mtDNA sequence databases appropriate for forensic use (JUST et al., 2011). Irwin et al. (2011) showed that a small set of coding region SNPs resolves a substantial proportion of the identical haplotypes. Moreover, this SNP set, while substantially increasing the discriminating efficiency in most Eurasian populations by roughly equal amounts, discloses population-specific profiles.

As more than 20% of our mtDNA population samples belong to haplogroup H, Real Time PCR SNP genotyping provides a simple, rapid and informative method to increase the discrimination power in cases of HV1/HV2/HV3 matches and proved to be very useful for forensic purposes. The advent of new methodological strategies for SNP typing, allied to the publication of complete mtDNA sequence population data, has given birth to a new phase in application of mtDNA to forensic casework, characterized by the incorporation of coding region information, like showed.

CONCLUSION

It was possible to conclude that coding region SNPs increase considerably the discrimination power of current mtDNA typing, specially the SNP 3010 for the haplogroup H. Currently, however, available forensic mtDNA reference databases only include information from mtDNA control region. Thus, future mtDNA databasing efforts are needed for the development of entire mtDNA genome reference population data suitable for forensic comparisons.

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Figure 1: Distribution of alleles of SNP 3010 after the samples classification in haplogroups Native Americans, Africans and Europeans in this work.

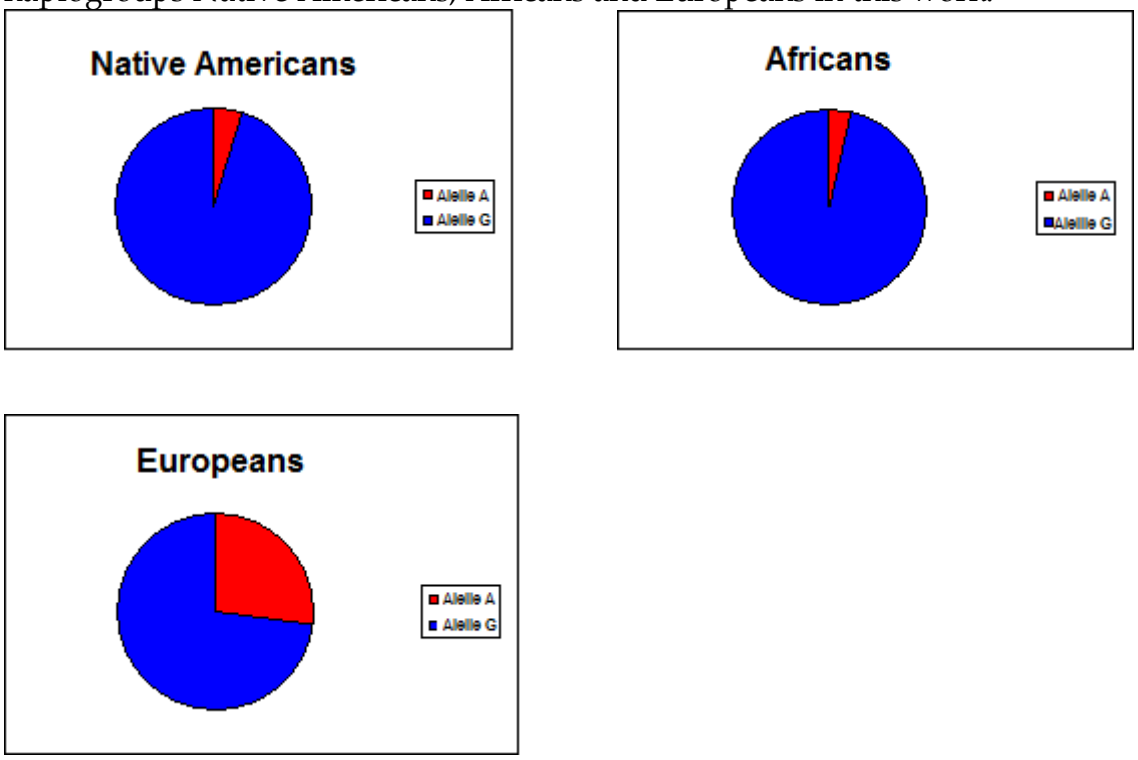

Table 1 – TaqMan Primers and Probes.

SNP 3010	**Sequences**
Forward Primer	5` – TTTACGACCTCGATGTTGGATCAG – 3`
Reverse Primer	5` – TCACGTAGGACTTTAATCGTTGAACAA – 3`
Probes	VIC – ACATCCC**G**ATGGTGC – NFQ FAM – ACATCCC**A**ATGGTGC – NFQ

Note: VIC and FAM are fluorophores, NFQ (Non fluorescent Quencher). Polymorphic nucleotides in the sequence probes are in bold and underlined.

Table 2 - Results of SNP 3010 genotyping in this study and different populations based on Human Mitochondrial Genome Database (mtDB).

Population	***Alelle A***	***Alelle G***
This work	10.63%	89.37%
Africans	4.41%	95.59%
Native-americans	20.96%	79.07%
Asians	27.70%	72.30%
Australians	8.82%	91.18%
Europeans	21.90%	78.10%
Total Media	**16.76%**	**83.24%**

Table S1: Mitochondrial DNA Control Region haplotypes and SNP 3010 in 160 individuals from São Paulo Metropolitan area, Brazil.

Sample	*HG*	**Control Region Haplotypes**													**SNP 3010**
BR-SP002	***C1d***	73G	152C	189G	194T	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	523DEL	524DEL	G
		16051G	16093C	16223T	16298C	16325C	16327T	16519C							
BR-SP004	***HV****	152C	263G	315.1C	523DEL	524DEL	16221T	16519C							A
BR-SP007	***L3e1a***	73G	150T	189G	200G	263G	309.1C	315.1C	16185T	16223T	16327T	16519C			G
BR-SP008	***X1***	73G	146C	153G	256T	263G	315.1C	16182C	16183C	16189C	16223T	16278T	16519C		G
BR-SP009	***J2***	73G	150T	152C	263G	295T	315.1C	489C	16069T	16126C	16193T	16278T			G
BR-SP010	***U3a***	73G	150T	263G	315.1C	16343G	16356C	16390A	16519C						A
BR-SP011	***B4b***	73G	263G	315.1C	499A	16126C	16183C	16189C	16217C	16372C	16519C				G
BR-SP015	***K2a***	73G	146C	152C	263G	315.1C	16224C	16311C	16519C						G
BR-SP016	***M****	73G	263G	309.1C	315.1C	489C	16192T	16519C							G
BR-SP017	***H3a***	152C	263G	309.1C	315.1C	16239G	16256T	16519C							G
BR-SP018	***L3e2b***	73G	150T	195C	263G	309.1C	315.1C	16172C	16183C	16189C	16193.1C	16223T	16320T		G
		16519C													
BR-SP019	***L1c***	73G	151T	152C	182T	186A	189C	195C	198T	204C	247A	263G	297G	309.1C	G
		315.1C	316A	459DEL	16037G	16187T	16189C	16223T	16271C	16274A	16278T	16291T	16294T	16311C	
		16360T	16519C												
BR-SP020	***L1b***	73G	152C	182T	185T	195C	228A	247A	263G	315.1C	523DEL	524DEL	16114G	16126C	G

		16187T	16189C	16223T	16264T	16270T	16274A	16278T	16293G	16311C	16519C				
BR-SP021	***L3e2b***	73G	150T	152C	195C	263G	315.1C	16172C	16183C	16189C	16223T	16239T	16320T	16519C	G
BR-SP022	***C1b***	71A	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16126C	G
		16223T	16270T	16298C	16325C	16327T	16438A								
BR-SP023	***B4b***	73G	103A	152C	263G	309.1C	315.1C	499A	16183C	16189C	16217C	16241G	16519C		G
BR-SP024	***L0a***	64T	93G	152C	189G	204C	207A	236C	247A	263G	309.1C	315.1C	523DEL	524DEL	G
		16148T	16172C	16187T	16188G	16189C	16223T	16230G	16311C	16320T	16519C				
BR-SP026	***L2b***	73G	150T	152C	182T	195C	198T	204C	263G	309.1C	315.1C	418T	523DEL	524DEL	G
		16114A	16129A	16213A	16223T	16278T	16325C	16355T	16390A						
BR-SP027	***A2***	59C	64T	73G	146C	152C	153G	204C	235G	263G	297G	309.1C	315.1C	523DEL	G
		524DEL	16126C	16223T	16278T	16290T	16319A	16362C							
BR-SP028	***L3e1***	73G	150T	189G	200G	263G	309.1C	315.1C	16223T	16327T					G
BR-SP029	***B4b***	73G	263G	309.1C	315.1C	499A	16158G	16183C	16189C	16193.1C	16217C	16394T	16519C		G
BR-SP030	***L2a1***	73G	143A	146C	152C	195C	198T	263G	315.1C	16187T	16189C	16192T	16223T	16278T	G
		16294T	16368C	16390A											
BR-SP032	***L2a1***	73G	143A	146C	152C	189G	195C	263G	315.1C	447T	16129A	16189C	16192T	16223T	G
		16278T	16294T	16309G	16390A										
BR-SP034	***A2***	73G	146C	153G	235G	263G	309.1C	315.1C	523DEL	524DEL	16111T	16126C	16223T	16259T	G
		16290T	16319A	16362C	16390A										
BR-SP035	***H***	263G	309.1C	315.1C	16311C										G

BR-SP036	***B2***	73G	263G	306DEL	307DEL	308DEL	309DEL	315.1C	499A	16173T	16182C	16183C	16189C	16217C	G
		16223T	16357C	16519C											
BR-SP037	***L3f1***	73G	150T	189G	200G	263G	309.1C	315.1C	16129A	16209C	16223T	16292T	16295T	16311C	G
		16519C													
BR-SP038	***L3e1***	73G	150T	152C	189G	200G	263G	315.1C	524.1A	524.2C	16176T	16223T	16327T		G
BR-SP039	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16153A	16223T	G
		16298C	16325C	16327T	16526A										
BR-SP040	***H5***	263G	282C	315.1C	456T	16294T	16304C	16320T							G
BR-SP041	***L2a1***	73G	143A	146C	152C	195C	263G	315.1C	16093C	16223T	16278T	16294T	16309G	16311C	G
		16320T	16390A	16519C											
BR-SP042	***L3e2b***	73G	150T	195C	263G	309.1C	315.1C	385G	523DEL	524DEL	16171G	16172C	16183C	16189C	G
		16223T	16320T	16519C											
BR-SP044	***L2a***	73G	146C	152C	195C	198T	263G	315.1C	523DEL	524DEL	16189C	16223T	16278T	16294T	G
		16309G	16390A	16519C											
BR-SP045	***L2a***	73G	146C	152C	195C	263G	315.1C	16182C	16183C	16189C	16223T	16278T	16290T	16294T	G
		16309G	16390A												
BR-SP046	***L3e2a***	73G	150T	195C	198T	263G	309.1C	315.1C	499A	16320T	16399G	16519C			G
BR-SP047	***H***	263G	315.1C	16093C	16519C										G
BR-SP050	***D4/G***	73G	146C	152C	195C	263G	309.1C	315.1C	489C	16172C	16220G	16223T	16241G	16311C	A
		16342C	16362C	16519C											

BR-SP051	***X2***	73G	146C	195C	263G	315.1C	16189C	16223T	16278T	16519C				G
BR-SP052	***J1***	73G	185A	228A	263G	295T	309.1C	315.1C	462T	489C	16069T	16126C	16519C	G
BR-SP053	***L3b***	73G	195C	263G	279C	315.1C	523DEL	524DEL	16066G	16145A	16223T	16278T	16362C	16519C	A
BR-SP054	***L1c***	73G	151T	152C	186A	189C	195C	198T	247A	263G	297G	315.1C	316A	523DEL	G
			524DEL	16078G	16129A	16187T	16189C	16223T	16265C	16286A	16294T	16311C	16320T	16360T	
			16519C	16527T											
BR-SP057	***L2b***	73G	146C	152C	182T	183G	195C	198T	204C	263G	309.1C	315.1C	16114A	16129A	G
			16213A	16223T	16274A	16278T	16390A								
BR-SP058	***H***	263G	315.1C	16172C	16210G	16519C									A
BR-SP059	***C1d2***	73G	194T	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	523DEL	524DEL	16051G	16223T	G
			16298C	16325C	16327T	16519C									
BR-SP060	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16223T	16298C	G
			16325C	16327T											
BR-SP061	***B4b***	73G	152C	263G	309.1C	315.1C	460C	499A	16092C	16183C	16189C	16217C	16468C	16519C	G
BR-SP063	***L1b***	73G	151T	152C	182T	185T	195C	247A	263G	315.1C	357G	523DEL	524DEL	16126C	G
			16187T	16189C	16223T	16264T	16270T	16278T	16311C	16519C					
BR-SP064	***T2***	73G	263G	309.1C	309.2C	315.1C	16126C	16241G	16294T	16304C	16519C				G
BR-SP065	***K1a***	73G	114T	150T	263G	309.1C	309.2C	315.1C	497T	16224C	16311C	16519C			G
BR-SP066	***L3f***	73G	146C	263G	309.1C	315.1C	374.1A	16038G	16209C	16223T	16294T	16311C	16519C		G
BR-SP067	***L3e2a***	73G	150T	195C	198T	263G	309.1C	315.1C	499A	16093C	16189C	16223T	16320T	16399G	G

		16519C												
BR-SP068	***A2***	64T	73G	146C	152C	153G	235G	263G	297G	309.1C	309.2C	315.1C	523DEL	524DEL	G
BR-SP070	***T1a***	73G	263G	309.1C	309.2C	315.1C	524.1A	524.2C	16126C	16163G	16186T	16189C	16284G	16294T	G
		16519C													
BR-SP071	***A2***	64T	73G	146C	153G	235G	263G	309.1C	309.2C	315.1C	384G	492G	523DEL	524DEL	G
			16111T	16223T	16290T	16311C	16319A	16356C	16362C						
BR-SP072	***L3e3***	73G	150T	195C	263G	309.1C	315.1C	523DEL	524DEL	16093C	16148T	16223T	16265T	16519C	G
BR-SP073	***H***	152C	263G	309.1C	315.1C										A
BR-SP074	***U5b1b***	73G	150T	152C	195C	263G	315.1C	16093C	16183C	16187T	16189C	16192T	16259G	16270T	G
BR-SP076	***L3e1***	73G	150T	189G	200G	263G	309.1C	315.1C	16183C	16189C	16223T	16260T	16327T		G
BR-SP077	***L2a1***	73G	146C	152C	195C	263G	309.1C	315.1C	16189C	16223T	16278T	16294T	16309G	16390A	G
		16519C													
BR-SP078	***L3e2b***	73G	150T	152C	195C	315.1C	16172C	16183C	16189C	16223T	16320T	16519C			G
BR-SP079	***L0a***	64T	93G	95C	152C	189G	236C	247A	263G	309.1C	315.1C	523DEL	524DEL	16093C	G
		16148T	16166G	16172C	16179T	16187T	16188G	16189C	16223T	16230G	16256T	16311C	16320T	16519C	
BR-SP080	***L3e1d***	73G	150T	152C	189G	200G	263G	315.1C	16176T	16223T	16256T	16327T			G
BR-SP082	***L3e1d***	73G	150T	152C	189G	200G	263G	315.1C	16176T	16223T	16327T				G
BR-SP083	***L3e1***	73G	150T	189G	263G	309.1C	315.1C	16223T	16327T						G
BR-SP084	***L1c2a1***	73G	151T	152C	182T	186A	189C	195C	198T	247A	263G	297G	309.1C	315.1C	G
		316A	16071T	16129A	16145A	16187T	16189C	16213A	16223T	16234T	16265C	16278T	16286G	16294T	

		16311C	16360T	16527T										
BR-SP085	***B2c***	73G	151T	260A	263G	309.1C	309.2C	315.1C	499A	523DEL	524DEL	16182C	16183C	16189C	G
			16217C	16234T	16316G										
BR-SP086	***C1b***	73G	152C	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16037G	G
			16223T	16294T	16298C	16325C	16327T								
BR-SP087	***K1a1a***	73G	263G	315.1C	497T	524.1A	524.2C	16093C	16224C	16311C	16519C				G
BR-SP089	***L3f1b4a***	73G	150T	189G	200G	204C	263G	309.1C	315.1C	16209C	16223T	16292T	16311C	16519C	G
BR-SP090	***A2***	64T	73G	146C	153G	210G	235G	263G	309.1C	315.1C	523DEL	524DEL	16111T	16209C	G
		16223T	16290T	16319A	16362C										
BR-SP091	***B2c***	73G	103A	152C	263G	315.1C	499A	16182C	16183C	16189C	16217C	16241G	16519C		G
BR-SP092	***B4b***	73G	263G	309.1C	315.1C	499A	16183C	16189C	16217C	16519C					G
BR-SP094	***L3b***	73G	195C	263G	279C	309.1C	315.1C	523DEL	524DEL	16145A	16223T	16278T	16362C	16519C	G
BR-SP095	***L3k***	73G	146C	150T	152C	235G	263G	315.1C	494T	16223T	16355T				G
BR-SP098	***B2c***	73G	103A	152C	263G	315.1C	499A	16182C	16183C	16189C	16217C	16241G	16519C		G
BR-SP099	***H***	263G	309.1C	309.2C	315.1C	16519C									G
BR-SP100	***L3d***	73G	152C	263G	315.1C	523DEL	524DEL	16124C	16183C	16189C	16223T	16278T	16304C	16311C	G
BR-SP101	***B2c***	73G	103A	152C	263G	315.1C	499A	16182C	16183C	16189C	16217C	16241G	16519C		G
BR-SP102	***D1***	73G	263G	315.1C	489C	16223T	16325C	16362C							A
BR-SP103	***L3e2***	73G	150T	195C	198T	263G	315.1C	499A	16223T	16269G	16320T	16399G	16519C		G
BR-SP106	***B2c***	73G	103A	152C	263G	499A	16182C	16183C	16189C	16217C	16241G	16519C			G

BR-SP109	***T2***	73G	263G	315.1C	16126C	16140C	16189C	16294T	16296T	16311C	16519C				G
BR-SP110	***L4b2***	73G	146C	152C	195C	244G	263G	309.1C	315.1C	340T	523DEL	524DEL	16051G	16114T	G
		16189C	16192T	16223T	16293T	16311C	16316G	16355T	16362C	16399G	16519C				
BR-SP111	***L3e1***	73G	150T	189G	200G	263G	315.1C	16172C	16223T	16327T	16399G				G
BR-SP112	***L2a***	73G	143A	146C	152C	263G	309.1C	315.1C	16189C	16193T	16193.1C	16223T	16278T	16294T	G
		16362C	16390A												
BR-SP113	***L3e1b***	73G	150T	185A	189G	263G	309.1C	315.1C	16223T	16325DEL	16327T				G
BR-SP114	***HV0***	72C	263G	309.1C	315.1C	16124C	16298C	16319A							G
BR-SP117	***L2a1a***	73G	146C	195C	263G	309.1C	315.1C	16189C	16192T	16223T	16278T	16294T	16309G	16390A	G
		16519C													
BR-SP118	***A2***	73G	146C	153G	235G	263G	315.1C	523DEL	524DEL	16111T	16126C	16223T	16259T	16290T	G
		16319A	16327T	16362C	16519C										
BR-SP119	***H5***	263G	309.1C	315.1C	456T	513A	523DEL	524DEL	16304C	16519C					G
BR-SP121	***L3f1b***	73G	200G	263G	309.1C	315.1C	16209C	16223T	16292T	16311C	16519C				G
BR-SP122	***L1c1a1***	73G	93G	151T	152C	182T	186A	189C	195C	247A	248G	263G	309.1C	315.1C	G
		316A	523DEL	524DEL	16129A	16187T	16189C	16223T	16278T	16293G	16294T	16311C	16360T	16519C	
BR-SP123	***A2***	64T	73G	146C	152C	153G	235G	263G	309.1C	315.1C	523DEL	524DEL	16092C	16111T	G
		16172C	16223T	16290T	16319A	16362C	16519C								
BR-SP124	***L1c2***	73G	151T	152C	182T	186A	189C	195C	198T	247A	263G	297G	309.1C	315.1C	G
		316A	16129A	16187T	16189C	16223T	16265C	16278T	16286G	16294T	16311C	16343T	16360T	16519C	

		16527T												
BR-SP125	***A2***	64T	73G	146C	153G	235G	263G	309.1C	309.2C	315.1C	374G	482C	523DEL	524DEL	G
		16111T	16215G	16223T	16290T	16319A	16362C	16519C							
BR-SP126	***L3e2b2***	73G	150T	195C	263G	315.1C	16172C	16182C	16183C	16189C	16223T	16320T	16519C		G
BR-SP127	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	513A	523DEL	524DEL	16223T	G
			16292T	16298C	16325C	16327T	16362C								
BR-SP128	***H***	152C	263G	315.1C	16519C										A
BR-SP129	***L2a1***	73G	146C	152C	195C	263G	309.1C	315.1C	16223T	16278T	16294T	16309G	16390A	16519C	G
BR-SP130	***L1c3a***	73G	151T	152C	182T	186A	189C	195C	247A	263G	309.1C	309.2C	315.1C	316A	G
		523DEL	524DEL	16093C	16129A	16183C	16189C	16215G	16223T	16278T	16294T	16311C	16355T	16360T	
		16390A	16519C												
BR-SP131	***L2b1a***	73G	150T	152C	182T	195C	198T	204C	263G	315.1C	418T	523DEL	524DEL	16114A	G
		16129A	16213A	16223T	16278T	16355T	16362C	16390A							
BR-SP132	***L2a1a4***	73G	146C	152C	195C	263G	309.1C	315.1C	316A	16223T	16278T	16286T	16294T	16309G	G
		16390A	16519C												
BR-SP133	***A2***	64T	73G	146C	235G	263G	309.1C	309.2C	315.1C	523DEL	524DEL	16111T	16223T	16290T	G
		16319A	16362C												
BR-SP134	***C1b***	73G	152C	249DEL	263G	290DEL	291DEL	309.1C	309.2C	315.1C	489C	493G	523DEL	524DEL	G
		16223T	16298C	16325C	16327T	16526A									
BR-SP136	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16223T	16298C	G

		16325C	16327T	16526A										
BR-SP137	***A2***	73G	146C	153G	235G	263G	315.1C	523DEL	524DEL	16092C	16111T	16223T	16290T	16319A	G
		16362C													
BR-SP138	***H***	93G	263G	309.1C	315.1C	16519C									A
BR-SP139	***L3e2b***	73G	150T	152C	195C	263G	309.1C	315.1C	16172C	16183C	16189C	16223T	16320T	16519C	G
BR-SP140	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	309.2C	315.1C	489C	493G	523DEL	524DEL	16223T	G
		16298C	16325C	16327T											
BR-SP142	***H***	263G	309.1C	315.1C	16311C	16519C									G
BR-SP143	***L0a1b***	93G	95C	185A	189G	236C	247A	263G	315.1C	523DEL	524DEL	16129A	16148T	16168T	G
		16172C	16187T	16188G	16189C	16223T	16230G	16278T	16293G	16311C	16320T				
BR-SP145	***L2a1***	73G	143A	146C	152C	195C	263G	309.1C	315.1C	16223T	16256T	16278T	16294T	16309G	G
		16390A													
BR-SP146	***H***	152C	263G	309.1C	309.2C	315.1C	16519C								G
BR-SP147	***N9b***	73G	150T	200G	263G	309.1C	309.2C	315.1C	16129A	16183C	16189C	16193.1C	16223T	16260T	G
		16327T													
BR-SP148	***H5***	93G	263G	309.1C	315.1C	456T	16294T	16304C							G
BR-SP149	***H***	263G	309.1C	315.1C	523DEL	524DEL	16519C								A
BR-SP150	***H5***	263G	309.1C	315.1C	456T	16294T	16304C	16320T							G
BR-SP151	***K1a4***	73G	263G	315.1C	497T	523.1A	524.1C	16129A	16224C	16311C	16519C				G
BR-SP152	***T1a***	73G	195C	263G	315.1C	16126C	16163G	16186T	16189C	16294T	16519C				G

BR-SP154	***U4a1***	73G	152C	195C	263G	309.1C	315.1C	499A	523.1A	524.1C	16134T	16240G	16286A	16356C	G
		16519C													
BR-SP155	***C1b2***	73G	152C	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16037G	G
		16223T	16298C	16325C	16327T										
BR-SP156	***J2b***	73G	150T	152C	263G	295T	315.1C	489C	16069T	16126C	16193T	16278T	16519C		G
BR-SP157	***J1***	73G	185A	188G	263G	295T	315.1C	462T	482C	489C	16069T				A
BR-SP158	***C1b***	73G	263G	249DEL	290DEL	291DEL	309.1C	315.1C	489C	493G	513A	523DEL	524DEL	16223T	G
		16292T	16298C	16325C	16327T	16362C									
BR-SP159	***L3f1b1***	73G	189G	195C	200G	263G	272G	309.1C	315.1C	16093C	16129A	16209C	16223T	16292T	G
		16295T	16311C	16519C											
BR-SP160	***L2a1***	73G	146C	152C	195C	263G	309.1C	315.1C	16189C	16223T	16278T	16294T	16309G	16390A	G
		16519C													
BR-SP161	***L3k***	73G	146C	150T	152C	200G	263G	309.1C	315.1C	494T	16188T	16223T			G
BR-SP162	***H***	152C	263G	315.1C	16519C										A
BR-SP163	***L2a1***	73G	146C	152C	195C	263G	309.1C	309.2C	315.1C	16189C	16192T	16223T	16274A	16278T	G
		16294T	16309G	16390A	16519C										
BR-SP164	***L4b1***	73G	150T	199C	204C	263G	309.1C	315.1C	513A	523DEL	524DEL	16179T	16182C	16183C	G
		16189C	16223T	16239T	16311C	16320T	16362C	16519C							
BR-SP165	***L0a1b***	93G	95C	185A	189G	236C	247A	263G	315.1C	523DEL	524DEL	16129A	16148T	16168T	G
		16172C	16187T	16188G	16189C	16223T	16230G	16278T	16293G	16311C	16320T				

BR-SP166	***H***	152C	263G	315.1C	524.1A	524.2C	16519C							G
BR-SP167	***L2a1***	73G	143A	146C	152C	195C	263G	309.1C	315.1C	16189C	16223T	16256T	16278T	16294T	A
		16309G	16390A											
BR-SP168	***H***	263G	309.1C	315.1C	16519C									A
BR-SP169	***L2a1***	73G	143A	146C	152C	195C	263G	315.1C	513A	16172C	16193T	16213A	16223T	16239T	A
		16278T	16294T	16309G	16390A									
BR-SP170	***M1a1***	73G	152C	195C	204C	315.1C	489C	16129A	16182C	16183C	16189C	16223T	16249C	16311C	G
		16359C	16519C	16527T										
BR-SP171	***B2c***	73G	263G	309.1C	315.1C	499A	523DEL	524DEL	16182C	16183C	16186T	16189C	16217C	16519C	G
BR-SP172	***J1***	73G	256T	263G	295T	309.1C	315.1C	462T	482C	489C	16069T	16126C			A
BR-SP173	***L0a2***	64T	93G	152C	189G	236C	247A	263G	309.1C	315.1C	523DEL	524DEL	16148T	16172C	G
		16187T	16188G	16189C	16223T	16230G	16311C	16320T	16519C						
BR-SP174	***H6***	239C	263G	309.1C	315.1C	16192T	16362C	16482G							G
BR-SP177	***H***	263G	315.1C	16311C	16519C										G
BR-SP178	***L2a1***	73G	146C	152C	195C	198T	263G	315.1C	16093C	16129A	16189C	16192T	16223T	16278T	G
		16294T	16309G	16390A										
BR-SP180	***L2a1b***	73G	146C	152C	195C	263G	315.1C	16182C	16183C	16189C	16223T	16278T	16290T	16294T	G
		16309G	16390A											
BR-SP181	***H***	263G	315.1C	524.1A	524.2C	16148T	16519C								G
BR-SP182	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16126C	16223T	G

		16298C	16325C	16327T											
BR-SP183	***U4a3***	73G	195C	247A	263G	315.1C	499A	523.1A	524.1C	525.1A	526.1C	16265G	16356C	16362C	G
		16519C													
BR-SP184	***L1b***	73G	152C	182T	185T	195C	247A	263G	315.1C	357G	523DEL	524DEL	16126C	16187T	G
		16189C	16223T	16264T	16270T	16278T	16293G	16311C	16317G	16519C					
BR-SP185	***W4***	73G	143A	189G	194T	195C	196C	204C	207A	263G	309.1C	315.1C	16223T	16286T	G
		16292T	16519C												
BR-SP186	***L2b***	73G	150T	152C	182T	195C	198T	204C	263G	315.1C	418T	523DEL	524DEL	16114A	G
		16129A	16213A	16223T	16278T	16355T	16390A								
BR-SP187	***L1c3***	73G	151T	152C	182T	186A	189C	195C	247A	248G	263G	315.1C	316A	523DEL	G
		524DEL	16129A	16187T	16189C	16223T	16278T	16293G	16294T	16311C	16360T	16519C			
BR-SP189	***L1c2***	73G	151T	152C	182T	186A	189C	195C	198T	247A	263G	297G	309.1C	315.1C	G
		316A	471C	523DEL	524DEL	16129A	16169T	16187T	16189C	16223T	16265C	16278T	16286G	16294T	
		16311C	16360T	16519C	16527T										
BR-SP190	***L2a1b***	73G	146C	152C	195C	263G	315.1C	16182C	16183C	16189C	16223T	16269C	16278T	16290T	G
		16294T	16309G	16390A											
BR-SP192	***B2c***	73G	103A	152C	263G	309.1C	309.2C	315.1C	499A	16182C	16183C	16189C	16217C	16241G	G
		16301T	16519C												
BR-SP193	***L2b1***	73G	150T	152C	182T	195C	198T	204C	263G	315.1C	418T	523DEL	524DEL	16114A	G
		16129A	16213A	16223T	16278T	16355T	16362C	16390A							

BR-SP194	***H***	263G	309.1C	315.1C	16184T	16263C	16265G	16519C						G
BR-SP195	***L3e1a***	73G	150T	189G	200G	263G	309.1C	315.1C	16185T	16223T	16327T	16519C		G
BR-SP196	***C1b***	73G	249DEL	263G	290DEL	291DEL	309.1C	315.1C	489C	493G	523DEL	524DEL	16126C	16223T	G
		16298C	16325C	16327T										
BR-SP197	***A2***	73G	146C	153G	235G	263G	309.1C	315.1C	16111T	16223T	16290T	16319A	16362C		G
BR-SP199	***L0a1b***	89C	93G	95C	185A	189G	236C	247A	263G	309.1C	315.1C	523DEL	524DEL	16129A	G
		16148T	16168T	16172C	16187T	16188G	16189C	16223T	16230G	16278T	16293G	16311C	16320T	16519C	
BR-SP201	***B4b***	73G	146C	152C	199C	263G	315.1C	499A	16183C	16189C	16217C	16519C			G

Note: HG (haplogroup)