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Two SNPs rs7786079 and a new one close to D7S820 microsatellite for subtyping African-American population

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Abstract. The design of new primers with the aim to obtain minialleles of microsatellites for forensic use is an important aim nowadays. This work started with that purpose: to design primers to reduce the length of D7S820 amplification products. After sequencing a long fragment around this microsatellite we discovered a new SNP (115upD7S820) not found in NCBI-SNP and http:www.ensembl.org databases. This new SNP was common in African-American individuals. The aim of this study is the differentiation between D7S820 alleles carrying the same number of repetitions based on these SNPs. © 2005 Elsevier B.V. All rights reserved.

Keywords: D7S820; Microsatellite; SNP; African-American population

1. Introduction

D7S820 microsatellite locus is usually found in commercial kits for paternity testing and individual identification. Our first intention was to design primers in order to reduce amplification product length which is very useful when using degraded DNA obtained from forensic samples. One of the primers designed matched with rs7786079 SNP [1], located 65 bp upstream D7S820 locus. Due to this mismatch in the 3′ end of the primer we were losing one allele or both of them in some samples.

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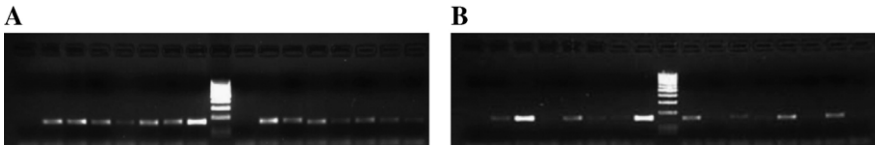

Fig. 1. Allele-specific amplification of African-American samples. (A) Amplification for A allele. (B) Amplification for C allele. Homozygotes and heterozygotes can be observed.

Sequencing a longer fragment which contained the microsatellite and the primer, we discovered that these African-American samples, showed another SNP which was not found in the databases. This new SNP is located 115 bp upstream the D7S820 and presents T and C alleles.

The next step was to study these two SNPs heterozygosities in African-Americans population and study if there is any linkage between them and D7S820 alleles.

2. Methodology

2.1. Samples analyzed

Sixty four African-American samples and thirty two Antioquian (Colombia) samples were analyzed. Purified DNA was quantified in a SmartSpec (BioRad) and diluted to a concentration of 10 ng/μl.

2.2. PCR amplification and SNP identification

rs7786079 SNP was studied with the primers designed for minialleles obtention using an allele-specific touchdown PCR with an annealing temperature of 63 °C and 57 °C. The fragment amplified was visualized in 2% agarose gels with ethidium bromide and UV illumination.

The new SNP was amplified by simple PCR program with an annealing temperature of 60 °C. Amplified products were visualized as described before. In this case, the PCR product was digested with *Sml*I restriction enzyme (NEB) and digestion products were visualized in 3% agarose gels with ethidium bromide and UV illumination.

2.3. Statistic analysis

Statistical calculations were performed with Gene Pop software (version 3.4).

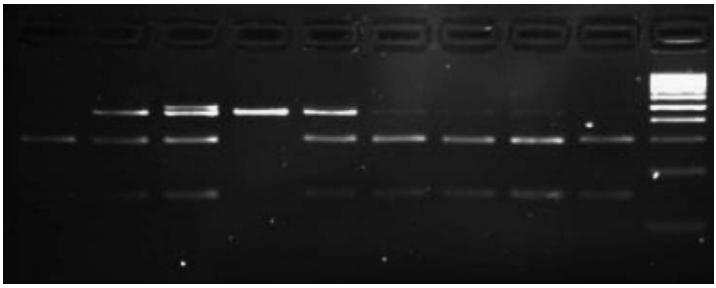

Fig. 2. *Sml*I digestion of PCR amplification products for the 115upD7S820 SNP.

Table 1
Contingency table for genotypic disequilibrium

rs7786079	New SNP			
	C/C	C/T	T/T	
A/A	40	1	0	41
A/C	1	8	0	9
C/C	1	3	4	8
	42	12	4	58

3. Results and conclusions

SNPs rs7786079 and 115upD7S820 were analyzed in two population samples, African-American and Hispanic from Colombia. The heterozygosity of rs7786079 was 0.198 in African-American sample, whereas the Hispanic Antioquia sample was A homozygous (some of the samples analyzed are shown in Fig. 1).

The 115upD7S820 SNP showed a heterozygosity of 0.167 in African-American sample and 0.019 in Antioquian individuals (Fig. 2).

These results indicate that the SNPs here studied are not discriminative of D7S820 alleles in Hispanic population from Antioquia. This is coincident with rs7786079 informativeness in Caucasian populations.

The disequilibrium test in African-American population sample has shown that these SNP loci are closely linked (Table 1), this linkage being A–C and C–T. These data are indicative that only one of both SNPs can be used to perform allelic discrimination between D7S820 alleles with the same number of repetition units.

In addition, these SNP loci do not show close linkage with D7S820 alleles, probably due to the higher mutation rate of the microsatellite loci. Taking this into account, it is possible to discriminate between D7S820 alleles combined with these SNPs. Moreover, subtyping locus D7S820 with one of this SNPs increases Discrimination Power from 0.805 to 0.934 in the African-American population group studied in our work.

In conclusion, the genetic identification based on D7S820 locus in African-American population could be improved combining the analysis of this microsatellite locus with the SNPs close to it.

Reference

[1] NCBI SNP Database (SSAHA: A Fast Search Method for Large DNA Databases).