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Development and forensic applications of multiplex PCR of autosomal biallele polymorphisms

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Abstract. The aim of this study was to set up a multiplex PCR of eight autosomal single nucleotide polymorphisms (SNPs) suitable for forensic purposes. © 2003 Published by Elsevier B.V.

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1. Introduction

The sequence of the human genome, within the framework of the Genoma Project, has revealed the existence of a new class of DNA polymorphisms involving one single base pair, called single nucleotide polymorphisms (SNPs), constituting an abundant form of genome variation [1,2]. On one hand, this new class of markers offers interesting perspectives in forensic casework, despite a considerable limitation: As they are biallele markers, there are only two different alleles in the population for each SNP, so that a large number of SNPs is required to achieve significant discrimination power [3,4]. On the other hand, SNPs may be used when DNA is highly degraded. SNPs allow us to analyze DNA regions not much longer than amplification primer pairs flanking the polymorphic site. The aims of this study were to set up a multiplex PCR of eight autosomal SNPs suitable for forensic purposes, to assay their discrimination power in a population sample and to compare it with the already known power of STRs commonly used in forensic work, and then to test them on degraded material. The multiplex PCR was constructed with eight primer pairs designed to produce amplicons in a range between 56 and 116 bp. One nanogram of DNA template extracted from 50 healthy Italian subjects and from 7 skeletal remains going back to the Second World War was submitted to amplification reaction. SNP typing was performed by minisequencing with the SnaPshot™ Multiplex Kit (AB). The extension products were electrophoresed in an automated five-colour capillary sequencer.

2. Materials and methods

Eight SNPs (Table 1), with an allele frequency of 0.5 in at least two different samples in a Caucasian population, were extrapolated from the database of ‘‘The SNP Consortium’’

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Table 1
SNPs selected, PCR primers, length of the relative amplicons and the type of polymorphism analyzed in our study

SNPs	Chromosome	PCR Primers 5′ → 3′	Amplicon length	Ambiguity codes
TSC 127227	Chr 12	GAGAGAATGCCAGTGTGGCTCA TGATTGGCCAGGTGGAGAACTC	56 bp	C/T
TSC 4055	Chr 22	TCCCATGCCCACTCACTTTACTA TGCACATGCCCAGTGTACTCA	76 bp	C/T
TSC 258676	Chr 20	ACTTCTCTGAGTCCACCAGACCTC TGCAAGAGAACAGATTGACCCA	81 bp	C/T
TSC 143414	Chr 16	CTATTTTCTGCATCTCCAAACCCC TGGTGGCAGAGGAGCTTCATA	82 bp	A/G
TSC 9926	Chr 15	AGATTCATCCCCAGGCTCTCA AAGGCTAGCTACCAGTGTCCACCA	85 bp	C/T
TSC 20329	Chr 19	CCCCTTCTGAAAAATGGTTTCACC TTCCGGAGAAAACAGTTCCCC	94 bp	G/T
TSC 21271	Chr 1	TTTCTTGTGGTAGGTGTCTGCCTC GCAAAGCAGCCCATCAGACAT	95 bp	A/G
TSC 249022	Chr 5	GCAGTGAGGCTGTGTTTGACAA GTCCAGAGATAGCTGAATTCCTCC	116 bp	A/G

(http://snp.cshl.org). DNA was extracted from the whole blood of 50 healthy subjects and from bones going back to the Second World War by phenol chloroform [5] and quantified by 1% agarose gel electrophoresis. Multiplex PCR primer sequences were designed by Primer Express™ 1.0 (AB). Table 1 shows the primers obtained and the length of the relative amplicons. One nanogram of genomic DNA was submitted to multiplex amplification in a final volume of 50 μl (PCR Buffer 1 ×, dNTPs mix 800 μM, $MgCl_2$ 2.5 mM, 2.5 U HotStarTaq DNA polimerase (Qiagen), primers 0.05 μM) in a GeneAmp®PCR System9 700 (AB) following these conditions: 95 °C for 15 min; 35 cycles at 94 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min; final extension at 72 °C for 10 min.

Polymorphic sites were detected by multiplex primer single-base extension reactions or minisequencing using the ABI PRISM® SnaPshot™ Multiplex Kit (AB) in the conditions recommended by the manufacturer. Primer extension products were separated by capillary electrophoresis on an ABI PRISM™ 310 Genetic Analyzer.

3. Results

All 50 subjects submitted to analysis showed 50 different genotypes, and this result supports the high polymorphisms of the selected markers. To investigate the discrimination power of SNPs, we calculated the match probability (Table 2) of each locus

Table 2
Probability that any two random individuals share the same genotype at each locus and at all loci

Locus	Match probability
TSC 21271	0.37868
TSC 9926	0.39361
TSC 249022	0.38560
TSC 20329	0.40891
TSC 258676	0.37754
TSC 4055	0.38004
TSC 127227	0.37590
TSC 143414	0.40891
Cumulative	0.00052

Table 3
Match probabilities of three commonly used markers and their cumulative match probability

Locus	Heterozygosity	Match probability
D7S820	0.793	0.07391
D13S317	0.767	0.08518
D5S818	0.723	0.12269
Cumulative		0.00077

(expressing the chance that two randomly chosen subjects have the same genotype). The product of these values is the cumulative match probability, or the probability of finding the same multilocus profile in any two random subjects. By comparison, Table 3 shows the match probabilities of three commonly used STRs. Positive results were obtained typing all the skeletal remains, where amplification of 15 common STRs used in forensic genetic (AmpFlSTR Identifiler PCR Amplification kit, AB) failed.

4. Discussion

This work shows that our multiplex PCR of autosomal biallele polymorphisms is robust and potentially valuable tools for forensic casework analysis. As SNPs are biallele markers, there are only two different alleles in the population for each one, so that a large number of SNPs is required to achieve significant discrimination power. Chakraborty et al. [3] and, later, Gill [4] have suggested that 30–60 SNP loci are necessary to match the power of the CODIS STR set, 13 STRs. This was confirmed by our study, which shows that three loci (D7S820, D13S317, D5S818) produce a cumulative match probability higher than that of the eight SNPs considered (i.e., they have less discriminatory power). A lower value can be obtained using four loci or, alternatively, three loci with higher heterozygosity.

In conclusion, the eight SNPs examined here provide a cumulative match probability similar to that obtained with three average STRs. Experiments on ancient DNA demonstrated that our markers seem to be useful in forensic casework when working with scarce and/or highly degraded material, in which common STRs fail.

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