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Nonbinary single-nucleotide polymorphism markers

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Abstract. Nonbinary single-nucleotide polymorphism markers (SNPs; i.e., comprising multiple substitutions) may be useful forensic markers as they offer increased discrimination and the possibility of mixture analysis. The NCBI dbSNP database was searched, and, out of the 13 markers selected, nine exhibited nonbinary polymorphism in African and European populations with expected allele distributions.

Keywords: Nonbinary; SNP; Forensic analysis

1. Introduction

Amplification of single-nucleotide polymorphism markers (SNPs) offers the possibility to analyze degraded material with increased success compared to existing techniques. However, the necessary reduction in amplicon size increases the probability of contamination and of encountering mixed DNA patterns [1]. We have examined one possible approach to establish control assays for such events using nonbinary SNPs. These markers amplify with equivalent efficiency to binary SNPs but could indicate the presence of extraneous DNA through the detection of a third or fourth allele. A search of the principal public database: NCBI dbSNP [2] revealed ~7000 markers exhibiting one of four types of three-allele polymorphism. From this set, the 13 most informative loci were regenotyped using African and European samples. The initial results of the detection and analysis of simple (two-donor) artificial mixtures are presented.

2. Material and methods

2.1. Bioinformatics

The NCBI dbSNP database was searched using the term: [ALLELE] prefixed with each type of three-allele variation (type V: A/C/G; type D: A/T/G; type B: C/T/G; and type H:

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C/T/A) yielding 1885, 1852, 1753 and 1896 SNPs respectively. Each group was reduced using [HETEROZYGOSITY] with "25:65" setting a range 25–65%.

2.2. SNP genotyping

Forty Galician (N.W. Spain) and 40 Mozambican samples were used. PCR primers were designed to give a product size range of 65–120 bp. Multiplex amplifications comprised 400 μM dNTPs, 2 mM MgCl2, 0.4 U Taq Gold polymerase and 2 ng DNA. Cycling conditions were 95 °C for 10 min, 32 cycles of 94 °C for 30 s, 58 °C for 30 s and 70 °C for 30 s, with a final extension of 65 °C for 15 min. Primer concentrations ranged from 0.5 to 1.0 μM. SNaPshot Minisequencing used 1 μl of PCR product cleaned with 0.5 μl of ExoSAP-IT (Amersham Biosciences) with conditions: 96 °C for 10 s, 50 °C for 5 s and 60 °C for 30 s, for 25 cycles. Extension primers were designed with an average t_m of 60 °C.

Table 1
Allele frequency distributions of nine nonbinary SNP loci in European and African samples (n=40) ranked in approximate order of information content

refSNP ID	Population[n]	dbSNP frequencies			Observed frequencies			%Dp	Probability 3rd
		C	A	T	C	A	T		
rs2839675	European	0.46	0.08	0.46	0.54	0.26	0.20	77	33.3
	African				0.38	0.24	0.38	81	41.7
		G	C	T	G	C	T		
rs5030240	European	0.50	0.35	0.15	0.58	0.27	0.15	75	28.5
	African				0.16	0.28	0.56	75	29.6
		A	G	C	A	G	C		
rs385780	European	0.42	0.30	0.28	0.46	0.36	0.18	78	35.6
	African	0.25	0.42	0.33	0.64	0.31	0.05	66	11.4
		C	T	A	C	T	A		
rs3091244	European	0.62	0.33	0.05	0.54	0.34	0.13	75	27.3
	African	0.26	0.41	0.33	0.39	0.49	0.13	75	28.4
		C	G	A	C	G	A		
rs865577	European	0.59	0.41	–	0.54	0.40	0.06	70	16.4
	African	0.35	0.54	0.09	0.39	0.52	0.09	73	22.0
		G	C	A	G	C	A		
rs2069945	European	0.57	0.43	–	0.51	0.40	0.09	72	21.2
	African	0.76	0.24	–	0.86	0.11	0.03	40	2.6
		A	T	C	A	T	C		
rs140676	European	0.57	0.33	0.10	0.58	0.34	0.08	71	18.8
	African				0.67	0.31	0.02	61	4.0
		T	A	G	T	A	G		
rs4540055	European	0.83	0.17	–	0.77	0.20	0.03	54	4.6
	African	0.13	0.47	0.40	0.06	0.45	0.49	70	15.2
		C	G	A	C	G	A		
rs228958	European	0.68	0.32	–	0.65	0.35	–		–
	African	0.45	0.48	0.07	0.53	0.43	0.04	68	12.2

%Dp and probability of observing a third allele in a simple mixture are listed.

2.3. DNA mixture experiments

DNA samples were mixed at ratios ranging from 1:4 to 4:1 and then amplified and minisequenced using a simple but robust three-SNP assay combination.

3. Results and discussion

Out of the 13 SNPs analyzed, nine gave nonbinary polymorphisms, and in both populations studied, eight gave nonbinary polymorphisms as well. Table 1 shows a list of the allele frequencies obtained. Observed alleles were as expected from dbSNP data, and frequencies were in good agreement with those reported elsewhere. One of the other four loci displaying only binary variation shows a third allele that appears to be confined exclusively to diabetic probands. The combined discrimination index of the nonbinary SNPs found is 99.996% in Europeans (8 loci) and 99.995% in Africans (9 loci) equivalent to 11 perfect (0.5:0.5) binary SNPs. In addition, the probability of encountering a third allele in simple mixtures is relatively high when these loci are used in combination. Mixture experiments detected minor component peaks between 1:1 to 1:3 ratios, suggesting that SNaPshot assays can detect extraneous DNA when present at high enough proportions.

References

[1] P. Gill, Int. J. Leg. Med. 114 (2001) 204–210.
[2] http://www.ncbi.nlm.nih.gov/entrez/.