DNA FINGERPRINTING WITH PROBES 33.15 AND 33.6 IN POPULATION FROM THE BASQUE COUNTRY

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INTRODUCTION

Some minisatellite areas dispersed through the human genome show a high level of genetic variability which makes them useful for identity and paternity purposes. This great variability in the number of repetitions among loci from different individuals, determines the probability of finding two unrelated people with an identical restriction profile to be minimum (Jeffreys et al. 1985a, Jeffreys et al. 1985 b).

As the application of these probes gives a high power of resolution, we have chosen multilocus probes 33.6 and 33.15 to carry out a study on a sample of resident population from the Basque Country. The obtained results will enable us to use these probes for Legal Medicine purposes in our population.

MATERIAL AND METHODS

The studied sample consisted of 50 unrelated individuals. Eight micrograms from each individual were digested with Hinf I and the obtained fragments fractionated by electrophoresis in 0.7% agarose gels. DNA from K562 cell line was used as control. The multilocus probes were labeled by the repeat unit multipriming system (Cellmark Diagnostics), using α^{32}P dGTP. The mobilities of the restriction fragments were measured using a videodensitometer.

RESULTS AND DISCUSSION

The average number of bands per individual obtained with probes 33.15 and 33.6 is shown in Table 1. Fragments were classified according to different size ranges to analyze them in the size class which gave the best resolution for each probe. Our results show that probe 33.15 resolves more fragments within the region of lowest size (in kb), while probe 33.6 reveals more fragments in the highest size class.

The probability of bandsharing between two unrelated individuals ($2q-q^2$) is shown in Table1. These results are similar to those obtained by Jeffreys et al. (1985c) and Gill et al. (1987) who previously found the probability of sharing one band to be higher for the smallest minisatellite fragments.

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The band-sharing distribution between two individuals fits a binomial distribution for both probes individually ($X^2_{5\ d.f} = 2.822$ for 33.15 and $X^2_{5\ d.f} = 2.579$ for 33.6). Both probes show very high heterozigosities (Table 2), which points out that they are extremely resolutives for identity and paternity testing purposes, since the combination of both probes reveals that the probability of all bands matching between two unrelated individuals is 1.403×10^{-21}.

Table 1. Similarities of DNA fingerprints between random pairs of individuals, using multilocus probes*

DNA fragments size (kb)	Fragments per individual ± s.d.	$2q-q^2$	q
PROBE 33.15			
23.13 - 9.42	1.6 ± 1.1	0.20	0.10
9.42 - 6.56	3.0 ± 1.3	0.29	0.14
6.56 - 4.37	7.2 ± 1.6	0.21	0.10
Mean no. of bands: 11.8 ± 2.6			
PROBE 33.6			
20 -10	4.3 ± 1.5	0.48	0.24
10 - 6	3.4 ± 1.5	0.34	0.17
6 - 4	6.2 ± 2.0	0.18	0.09
Mean no. of bands: 13.9 ± 3.9			

* Data were obtained from a sample of 43 unrelated Basque Country individuals

Table 2. Mean allele frequencies (q), heterozygosities (H), and mean probability of two unrelated individuals sharing all fragments ($2q-q^2$), detected by probes 33.15 and 33.6

	PROBE 33.15	PROBE 33.6	33.15+33.6
q	0.087	0.103	0.095
H	0.913	0.897	0.905
$2q-q^2$	7.674 E-10	1.829 E-10	1.403 E-21

The band-sharing distribution between two individuals follows a binomial distribution for both probes individually ($X^2_{5d.f.} = 2.822$ for 33.15 and $X^2_{5d.f.} = 2.579$ for 33.6). Both probes 33.15 and 33.6, show very high heterozigosities.

In order to test the exclusion power of these probes in paternity testing, 5 trios mother-child-alleged father selected among those we have been requested to resolve until now were studied. The results obtained from the DNA fingerprints in each paternity case are shown in Table 3. In cases 1, 3 and 5, bandsharing between the mother and the child was that expected (≈62,6%). However, the children did not show any father-specific band, excluding case 5, in which the child showed only one father-specific band.

In cases 2 and 4, the expected mother-child bandsharing was confirmed. A high number of shared-bands between alleged fathers and children was also found. In case 2, all the child´s bands were assigned, and in case 4 there remains just one unassigned band, which is supposed to be a mutant one. As the data did not exclude the alleged fathers, probe 33.6 was also applied to cases 2 and 4. The use of this probe resulted in the band assignaments shown in Table 3. Also in this table, the calculated probabilities of paternity for each probe and for each mother-child-alleged father trio are summarized. The analysis of these five cases studied agreed to the results previously obtained through the study of the conventional markers (table 4). In all the cases where these markers had excluded paternity, probes 33.15 and 33.6 also did it. This exclusion was particularly evident in case 5 where after the analysis of 19 protein systems and red cell groups, exclusion could only be stated by the HLA system. The probabilities of paternity obtained with multilocus probes 33.15 and 33.6 were much higher than those obtained through conventional markers. This makes the application of these probes to be of great interest also in the Basque Country.

Table 3. Assigned bands and Pp in paternity cases using probes 33.15 and 33.6

Case	Parents' shared bands	Child's bands: Mother specific	Father specific	Shared bands	Unassigned bands	Pp
Probe 33.15						
#1	3	6	0	3	6	
#2	3	6	5	3	0	0.9998
#3	4	5	0	4	7	
#4	1	6	3	1	1	0.6992
#5	4	5	1	2	8	
Probe 33.6						
#2	10	2	3	8	0	0.7574
#4	4	7	10	4	0	0.9999
Probes 33.15+33.6						
#2	13	8	8	11	0	0.999943
#4	5	13	13	5	1	0.999991

Table 4. Results in five cases tested with conventional systems

CASE	SYSTEMS WITH OBSERVED EXCLUSION	Pp
#1	MNSs, Gc, PGM1, ADA	
#2	Not observed	0.952
#3	MNSs, GLO, AK	
#4	Not observed	0.996
#5	HLA A, B, C	

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