MULTILOCUS PROFILES OF THE BASQUE COUNTRY POPULATION WITH DIG-LABELLED PROBES 33.15 AND 33.6

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INTRODUCTION

Probes 33.15 and 33.6 are frequently used due to their high discrimination power. Usually methods for labeling these multilocus probes are based on radioactive isotopes. The main disadvantages of this labeling-technique are the short half-life of the isotopes, the requirement of safety facilities, and the manipulation risk. For these reasons, probes labeled with molecules such as biotin or digoxigenin (DIG) have emerged as practical alternatives to radiolabeled probes (1).

We have developed a method to obtain DNA fingerprints with multilocus probes 33.15 and 33.6 employing the combination of non-isotopic labeling techniques by enzimatic incorporation of DIG-11-dUTP and chemiluminescent developing. These so labeled probes were also applied to a population sample of 30 unrelated individuals from the Basque Country in order to compare the discriminative power with ^{32}P labeled probes.

MATERIALS and METHODS

DNA from individuals were extracted by the phenol-chloroform method. Human DNA (8 μg) was digested with 30 units of Hinf I and the fragments were separated in 0.7% agarose gels with TBE 1x buffer at 3 V/cm until the 2.3 kb λ Hind III fragment migrated 18 cm. Also, in order to achieve accurate DNA fragments sizing, a ladder was loaded within each gel. Fragments were transferred to nylon membrane (Boehringer-Mannheim) using Southern's method.

Probes 33.15 or 33.6 (DNA inserts) were labeled as follows: 8 ng of probe made up to 20 μl with H_2O-MilliQ and denaturated, 4 μl of 10x Hexanucleotide mixture, 4μl of dNTPs (containing the Boehringer-Mannheim DIG-labeled dUTP), 4 μl of 2M Hepes buffer pH 6.6, 4 μl of labeling buffer (0.05M $MgCl_2$, 1M Tris-HCl pH 8.0) and 4 μl of 2U/μl Klenow enzyme were mixed and incubated at 37 °C o/n. The probe was precipitated with 4 μl of 8M LiCl and 110 μl of prechilled (-20 °C) ethanol, and left at -80 °C for 1 h. After centrifugation for 20 min at 15000 g, the pellet was washed with 110 μl of prechilled 70% ethanol, centrifugated for 5 min at 13000 g and dissolved in 40 μl of TE buffer o/n at room temperature. The labeled probe was further purified using a Sephadex G-50 column. The eluate was collected in Eppendorf tubes (3 drops in each one). Five μl of each eluted fraction were removed to prepare a slot-blot. Those fractions showing the maximum intensity (which contain the purified probe) by means of the DIG-luminescent detection described below, were mixed. The purified probe may be used directly for hybridization or stored at -20°C.

In order to label the ladder probe, 22 μl of 0.4 μM DNA marker probe (BRL DNA

analysis marker system) were treated in the same way as described above except that it was not denaturated and the hexanucleotide mixture was not added.
The membrane was prehybridized at 50 °C for 45 min in 0.5 M Na_2HPO_4 (pH 7.2) and 1% SDS. Hybridization was carried out at 62 °C overnight with 20 pg of denaturated DIG-labeled probe 33.15 or 33.6 and 0.1 µl of ladder DIG-labeled probe in 0.04 ml of hybridization solution/cm^2 of membrane. The hybridization solution used was the same composition as in the prehybridization but including a 10% v/v blocking reagent (casein in 0.1 M maleic acid buffer); after hybridization the solution was removed and stored at -20 °C to reuse. In order to remove the excess of the probe, the membrane was washed for 30 min in 0.2 M Na_2HPO_4 (pH 7.2) and 0.1% SDS at 62 °C.
The DIG Luminescent detection was carried out as in the Boehringer-Mannheim protocols with modifications: (1) the equilibration step was performed with 0.1 M maleic acid and 0.15 M NaCl pH 9.5; (2) the antibody incubation step was performed with 0.1 M maleic acid and 0.15 M NaCl pH 7.5 and without blocking reagent; (3) chemiluminescent detection was carried out with Lumiphos 530™ sprayed onto the membrane (0.01ml/cm^2); (4) an X-ray film was exposed to the membrane for 2-3 h at 37 °C using amplifying screens.
Statistical analysis of data was as described by Jeffreys et al. (2).

RESULTS and DISCUSSION

The labeling procedure does not require to remove the non-incorporated DIG-dUTP because it does not bind to membrane or DNA and does not create background. However, we have observed that the purification of the labeled probe with Sephadex G-50 column reduce the unspecific hybridization. This can be due to the elimination of the small DIG-fragments generated during the labeling procedure of these probes, formed by unions head-and-tail of the core sequences.
DNA fingerprints were obtained from a sample of 30 unrelated individuals from the Basque Country. The bands of every sample were analysed in the range within 20-4 kb (figure 1). The average number of bands per individual obtained with DIG-labeled probe 33.15 is 16.4 ± 2.87 in the 20-4 kb size range and probe 33.6 detecs 19.6 ± 3.03 bands in the same range (table 1). Previous data (2,3,4) show a lower average number of bands when probes 33.15 and 33.6 are radiolabeled. The comparison between the number of fragments resolved by DIG-probes and ^{32}P probes shows that DIG-probes detect more fragments in every region being the highest increase in the region 20-10 studied with the probe 33.15.
The bandsharing probabilities between two unrelated individuals are also lower than those for ^{32}P probes in every studied region and therefore the mean probability of sharing one band is also lower for both DIG-probes (0,187 for probe 33.15 and 0.192 for probe 33.6). The bandsharing distribution between two individuals fits a binomial distribution for both probes individually (X^2_{3df} = 0.830 for 33.15 and X^2_{3df} = 1.176 for 33.6, P > 0.05). Both probes show very high heterozygosities (table 1), which points out that they are extremely resolutive for identity purposes, since the mean probability of all bands matching between two unrelated individuals is 5.695×10^{-13} for probe 33.15 and 1.115×10^{-14} for probe 33.6.

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In summary, the method proposed here for those probes detecting minisatellite regions is reliable, reproducible and considerably facilitates its utilization because of a number of advantages including simplicity, operational advantages, speed, safety, and a higher resolution.

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Table 1. Probabilities of sharing one band in every region between random pairs of individuals (p), mean probability of two unrelated individuals sharing all fragments (X) and heterozygosities (H) detected by multilocus DIG-probes 33.15 and 33.6.

DNA fragments size (kb)	Fragments per individual ± s.d.	p	H
Probe 33.15			
zone I (20-10 kb)	4.1±1.4 (2.9±1.0)	0.162	0.919
zone II (10-6 kb)	4.8±1.3 (5.1±1.1)	0.178	0.911
zone III (6-4 kb)	7.9±1.3 (6.7±1.2)	0.205	0.897
Mean nº of bands 16.4 ± 2.9, X = 5.7×10^{-13}			
Probe 33.6			
zone I (20-10 kb)	4.6±1.7 (2.8±1.0)	0.168	0.916
zone II (10-6 kb)	5.6±1.5 (5.1±1.3)	0.190	0.905
zone III (6 -4 kb)	9.3±1.3 (6.7±1.2)	0.206	0.897
Mean nº of bands: 19.6 ± 3.0, X = 1.1×10^{-14}			

Figures in parenthesis give results by (2)

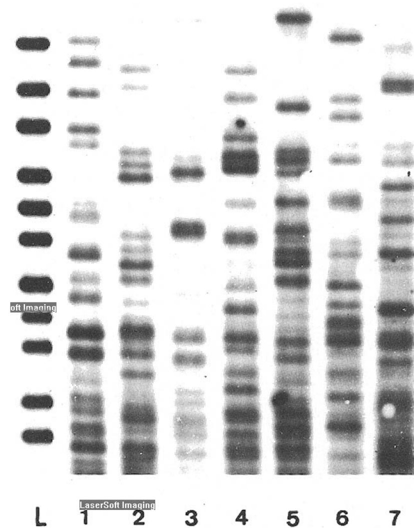

Figure 1.- Hybridization of the DIG-labeled probe 33.15 to Hinf I-digested genomic DNA samples of seven unrelated individuals (1-7) to the 4 kb cut off point. (L) ladder.

REFERENCES

(3) Gill, P., Lygo, J.E., Fowler, S.J. and Werrett, D.J., Electrophoresis 1987, 8, 38-44.
(2) Jeffreys, A.J., Wilson, V. and Thein, S.L., Nature 1985, 316, 76-79.
(1) Holtke, H.J., Sagner, G., Kessler, C. and Schmitz, G., Biotechniques 1992, 12, 104-13.
(4) Alonso, S., Castro, A., García-Orad, A., Arizti, P. and M. de Pancorbo, M., Advances in Forensic Haemogenetics, 1992, 4, 231-233.

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