VNTR Polymorphism: Reproducibility in techniques and interpretation

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Variable number of tandem repeat (VNTR) markers, first described by Wyman and White (1980), are highly polymorphic DNA regions of great value for paternity and forensic investigations. The polymorphism is displayed by the relative migration of individual bands, alleles, according to the number of repeats. Some highly polymorphic VNTR systems include alleles so close to each other in size that they are difficult to distinguish in RFLP. In order to determine the technical reproducibility and the reliability of the interpretation of such systems duplicate DNA samples on parallel electrophoresis were analyzed with the probe pYNH24. The size of the fragments was calculated and the results compared.

MATERIALS AND METHODS

DNA was prepared from 461 blood donors according to the SDS-urea method described by Lindblom and Holmlund (1988). Two series of 3 ug DNA from each blood sample were cleaved with 6-9 units Hinf I (Boehringer Mannheim, West Germany) and the fragments separated in parallel electrophoresis. Four lanes with fragment size markers (10 ng of Hind III and 15 ng of Eco RV cleaved Lambda DNA) and one lane with a genomic DNA reference were included on each gel. The DNA was transferred to Zeta-Probe membranes (Bio-Rad Laboratories, USA) by vacuum blotting (VacuGene, Pharmacia-LKB, Sweden). The fragment size markers were visualized by adding 20 ng Lambda DNA to the probe pYNH24 (Nakamura 1987) before radioactive labelling. Hybridization and washes were performed under normal stringencies. The autoradiograms were analyzed by image processing using a computer system (LabEye DNAE, Innovative Vision AB, Linköping, Sweden), by which the size of the individual fragments was calculated according to the fragment size markers included. For these calculations four bands (9 416, 5 376, 2 884 and 1 921) in each size marker lane had to be clearly visible for the results to be included in the study. The distribution of "alleles" was analyzed using the logarithm of the number of base pairs (bp) calculated for each fragment.

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RESULTS

The genomic DNA reference was analyzed 41 times and the hybridization with pYNH24 gave two bands, one within the range of 7 211 - 7 638 bp and the other within 2 823 - 2 927 bp with means and standard deviations of 7 352 $\pm$ 96 and 2 864 $\pm$ 27 respectively. Thus the standard deviations were approximately 1 % of the size of the fragments.

In 186 DNA duplicates the fragments were compared by linear regression analysis and the correlation coefficient was r = 0.999. The scattergram of these duplicates is shown in Fig. 1.

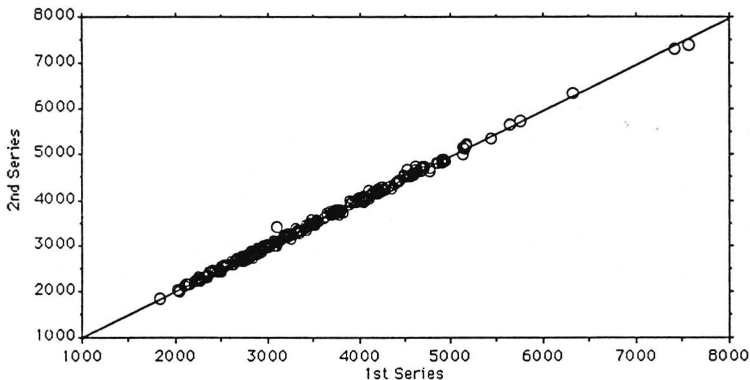

Fig. 1. Linear regression analysis of fragment sizes (bp) defined by pYNH24 from 186 DNA saples tested in parallel.

The allelic distribution was analyzed in 326 single tested samples. The bimodal distribution is shown in Fig. 2. 294 (90 %) of the tested individuals were heterozygous with two clearly separated bands. The most frequent "allele" had the size of 4 000 - 4 100 bp and a gene frequency of 0.077.

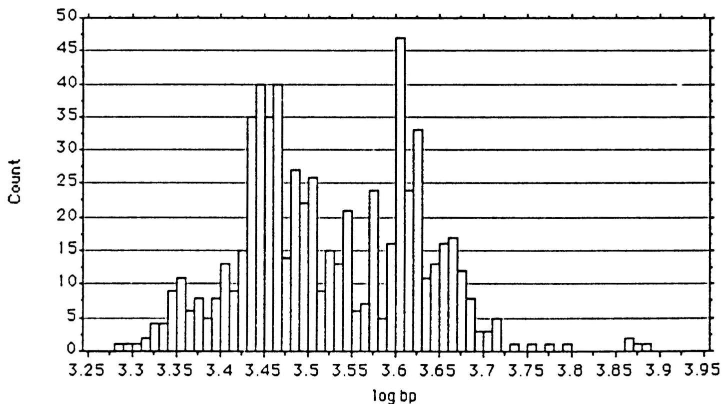

Fig. 2. Distribution of fragments (log bp) defined by pYNH24 in 326 blood donors.

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DISCUSSION

The aim of this study was to see if VNTR polymorphisms from different electrophoresis can be compared. For this purpose the size of the fragments defined by pYNH24 were calculated by image processing using size markers. The results from 186 blood donors out of the 461 tested could be compared. The parallel analysis of the other 275 samples were not comparable because of a weak hybridization signal with the smallest fragment in the size marker. This problem could be solved with size markers where the number of copies for each fragment increases with decreasing size. However, the linear regression analysis of the 186 duplicates has a correlation good enough (r = 0.999) to make conclusions about samples tested on different electrophoresis.

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