Forensic DNA-typing in the Netherlands using VNTR Single Locus Probes

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Introduction

Variable number of repeat (VNTR) base sequences are one of the most informative genetic DNA-polymorphisms for identification purposes. We have implemented the analysis of VNTR-loci at the Netherlands Forensic Science Institute using the Single Locus Probe hybridization technique. This technique is essentially the same one as agreed upon by the European DNA Profiling (EDNAP) group *[3]*. The forensic use of this technique is regarded as valid and reliable *[1,2]*. However, interpretation of the test results is highly dependent on the population genetics of the various DNA-markers and the forensic validation of this DNA-test in the Netherlands requires:

1. the implementation of match criteria for the comparison of DNA-profiles.
2. the collection of allele frequency data from the relevant population(s).

To this end allele frequency distributions of four different VNTR loci (D1S7, D2S44, D12S11 and D7S22) were collected in a population sample of 275 Dutch Caucasians. The criteria for the evaluation of matching samples were obtained from the interlab variations in allele size measurements of matching samples and K-562 genomic control DNA.

We have also evaluated the actual independence of VNTR allele-frequency data within the four different loci. With the results of this study an allele frequency of an estimated fragment is calculated by counting the number of alleles from the population database within the 2 extremes of the set window.

Methods

Population sample

Blood was obtained from unrelated male and female Dutch Caucasian donors.

Electrophoretic system and DNA-probes

Hinf I digested DNA was electrophoresed according to the EDNAP protocol *[3]* and Southern blotted onto Nylon membranes. Membranes were hybridized according to the manufactures instructions with synthetic oligonucleotide VNTR-probes which were already labeled with alkaline phosphatase.

The DNA-fragments were detected by chemiluminescence.

Analysis of band positions

The length of the DNA-fragments was calculated by reference to the DNA-analysis system (BRL) using the Pharmacia Gelscan XL laserscan with the Gelscan XL (version 2.1) software. HinfI restricted K562 genomic control DNA was included on every analytical gel.

Statistics

Allele-frequencies were estimated for each VNTR-locus by counting the number of fragments within a given interval and dividing by the total number of alleles in the database. The estimation of the allele frequencies was highly facilitated by using the Excel 4.0 frequency function. The homozygote frequency for each locus was obtained by counting the number of individuals with a one fragment pattern.

We tested for deviations from Hardy Weinberg equilibrium using the quantile chi-test proposed by Geisser and Johnson *[4]*.

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Results

Matching criteria

The quantitative aspects of our matching criteria were derived from repetitive measurements of human DNA-samples, repetitive measurements of the K562 cell line DNA which runs as a control on every analytical gel and from within-autolumigraph comparisons of DNA from stains from different sources (blood, semen and vaginal fluid) and DNA from liquid blood of the victim c.q. the suspect.

Table 1 shows the relevant data on the K562 DNA for four VNTR loci. The K562 allele sizes are monitored contineously as a QC measure. Except for the 13410 bp fragment for locus MS43A (CV=0.95%) the coefficients of variation (CV) for the K562 alleles were below 0.5%.

The average difference between DNA extracted from stains and the reference DNA comes to -0.26% which indicates that samples which contain degraded DNA tend to migrate faster than undegraded DNA-samples.

About 90% of the band comparisons were within 1.0%, 8% of the band measurements exceeded ± 1.0% and 2% of the comparisons exceeded ±2%. No comparison exceeded ± 2.5%.

These results show that we can safely compare and identify our DNA profiles on the ±2.8% matching guideline which has been proposed by Berry and Evett [5].

Population Genetics

The predefined conservative matching guide line (±2.8%) was used to generate the allele frequency histograms for the VNTR loci. The bin sizes in the histograms are defined by: bin-size = {0.056*fragment length}.

Allele frequency profiles of each system are reported in figures 1a-1d.

From the genotype distributions (not shown) it was concluded that there is little if any correlation between the paternal and maternal fragment lengths in the 4 VNTR loci. We actually tested for deviations from Hardy-Weinberg equilibrium using the quantile chi²-test [4]. Our test results did not indicate deviations from H-W equilibrium for either of the four loci.

Table 2 shows the observed homozygosity, the most common alleles and the most common genotype-frequencies of the four loci.

Exact comparisons with other Caucasian population samples could not be made. However the shapes of the frequency distributions found in this study are comparable with those reported for other Caucasian population samples.

Conclusion

SLP DNA Profiling has been successfully applied over the last three years at the Netherlands Forensic Science Laboratory at providing strong evidence from seminal stains and bloodstains.

Combined with other extensive validation studies this population genetic study will allow the typing of VNTR-loci to be used in forensic identity testing in the Netherlands.

The inter- and intragel variations of fragment length measurements in terms of basepairs were investigated. The variation in terms of % basepairs between duplicate measurements of control DNA and between DNA obtained from blood and stain-material was constant in the region 2-8 Kbp but increased slightly in the region of fragment lengths over 10 Kbp. Although the set window of ±2.8% is highly conservative, especially in the region of 2-8 Kbp it is highly convenient to use a window of constant width in terms of % basepairs.

We have found no evidence for the relationship between the paternal and maternal fragment lengths in any of the four loci. These results allow the use of the 2pq rule to calculate genotype frequencies where p and q are the respective allele frequencies.

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Table 1 K562 allele length data for four VNTR's.

VNTR-locus	D1S7		D2S44		D7S22		D12S11	
DNA-probe	MS-1		YNH-24		g3		MS43A	
K562 base-pair	allele size (bp)		allele size (bp)		allele size (bp)		allele size (bp)	
measurements	long	short	long	short	long	short	long	short
Average (bp)	4820	4450	4000	2890	7050	1970	13410	5260
Maximum (bp)	4870	4510	4030	2920	7100	1990	13780	5300
Minimum (bp)	4780	4400	3980	2870	6990	1960	13150	5220
1 SD	18	20	12	10	26	8	128	18
CV	0.36	0.46	0.30	0.35	0.37	0.40	0.95	0.34

Table 2 Homozygosity, maximal allele frequencies and maximal genotype frequencies of 4 VNTR loci in the Dutch Caucasian population.

VNTR-locus	D1S7	D2S44	D7S22	D12S11
DNA-probe	MS-1	YNH-24	g3	MS43A
N database	268	275	200	267
% Homozygosity	3.36	7.64	6.00	6.37
bp of max allele frequency	4860 5720	2820	1720	8840
max allele freq (%)	4.66	14.73	13.50	15.54
max genotype freq (%) *	0.43	2.88	2.29	4.42

*: expected maximum genotype frequency (2pq rule)

Table 3 P-values of the quantile chi²-test, using 4 classes

VNTR-locus	P-value
D1S7	0.52
D2S44	0.11
D7S22	0.54
D12S11	0.76

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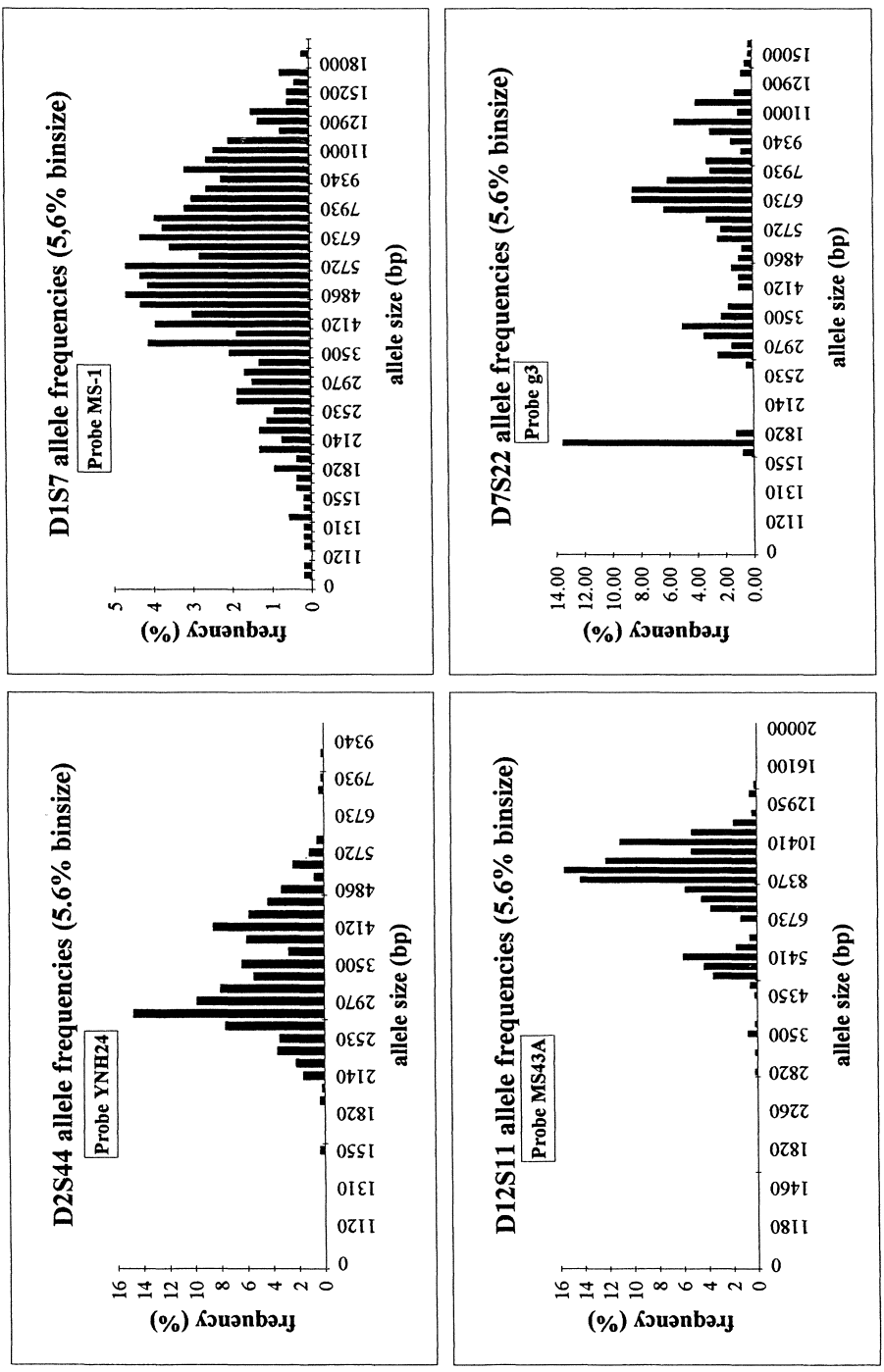
Figure 1
VNTR allele frequencies in the Dutch Caucasian population



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