TWO HIGHLY POLYMORPHIC VNTR LOCI D5S110 (LH1) AND D4S139 (PH30): ANALYSIS, FORMAL AND POPULATION GENETIC DATA.

C. Luckenbach*, A. Luckenbach*,V. Almeida, M. Mainka*, J. Jung*, H. Ritter***

* Inst. f. Anthroplogie und Humangenetik, Wilhelmstr. 27, 72074 Tübingen, FRG
**Inst. Anthropologia, Universidade Porto, 4000 Porto, Portugal

Summary
The experimental technique is described that enable RFLP typing with the two hypervariable VNTR loci D5S110 (LH1) and D4S139 (pH30). Population and formal genetic studies were performed using HaeIII restricted DNA from 450 unrelated individuals (SW-Germany) and 35 families with 172 children (NW-Portugal). Both loci reveal more than 70 alleles ranging between 0.8 kb and 9.5 kb at D5S110 (LH1) and between 1.5 kb and 19.5 kb at D4S139 (pH30). In 172 meioses analysed, one recombination event was observed at the D4S139 locus (recombination frequency of 0.0058) and none at the locus D5S110. Allele frequency distributions were determined for each of the two loci. Heterozygosity calculation results in 93.01% for LH1 and 96.62% for ph30.
The findings show that these loci represent further genetic markers which are very informative for identity tests as well as for parentage analysis.

1. Introduction
The most evident genetic markers for discrimination among individuals are the highly polymorphic variable number of tandem repeats (VNTR) loci. The technology to use these markers is the well-characterized restriction fragment length polymorphism (RFLP) analysis. In this study we describe two loci, D5S110 (LH1), Armour et al. (1990), and D4S139 (pH30), Milner et al. (1989), which are compatible with the restriction enzyme HaeIII used with other known VNTR loci like D2S44 (YNH24), D10S28 (TBQ7) and D17S26 (EFD52). Furthermore we present the frequency distributions of the alleles, the heterozygosity and the mutation rates for both loci.

2. Materials and methods

2.1. Experimental technique
DNA-Isolation: 5 ug human genomic DNA and cell-line K562 DNA according to the protocol of Miller et al. (1988).
RE-Digest: 50 U Hae III (Boehringer Mannheim / FRG).
Molecular weight marker: NICE™ DNA Analysis Ladder (GIBCO BRL, Eggenstein/FRG) with fragment sizes ranging from 22.621 kb to 0.526 kb.
Electrophoresis: 0.8% agarose gel (TBE buffer), 40V, 30hrs.
Transfer: Vacuum blotting with 50 mbar
Hybridization: Alkaline phosphatase-conjugated single-locus probe LH1 and pH30 (Gibco BRL) and the molecular weight marker probe MW100 (Gibco BRL)
Detection: Chemiluminescent with CSPD (Promega)

2.2. Image analysis and molecular weight calculation
according to Luckenbach et al. (1994)

2.3. Allele frequencies
Allele sizes are stored in a multi-user relational database and frequencies were analysed according to the classical mathematical rounding procedure (0.05kb-0.14kb = 0.1kb). 450 unrelated individuals from SW-Germany and 35 families with 172 children from N-Portugal were tested.

2.4. Statistical evaluations
We selected some common statistical paramters, i. e. arithmetic mean (AM), standard

deviation (SD) and coefficient of variation (CV) to check the precision of our analysis method. Thus we have made serial intergel measurements of the two K562 DNA fragments separated on 35 gels and hybridized to LH1 and pH30.

3. Results and Discussion

3.1. Gel Image

Figure 1 shows examples of the fragment patterns of HaeIII restricted DNA from 16 individuals and the cell-line K562 DNA, detected by LH1 and pH30; the molecular weight markers run in lane 1, 10 and 19. These profiles represent sharp bands ranging from 6 kb to 1kb (LH1) and from 15 kb to 3 kb (pH30). Each person is heterozygous with only one exception.

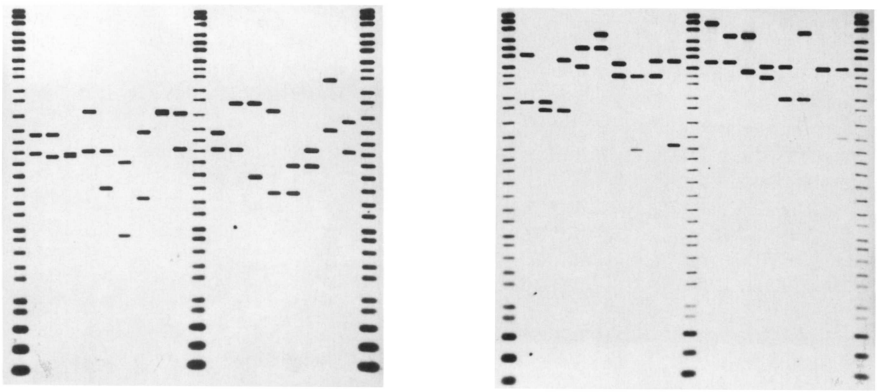

Fig.1: Image pattern of HaeIII restricted DNA from 16 individuals, (lanes 2-9, 11-18), the K562 cell-line (lane 19), detected by LH1and pH30, molecular weight marker MW100 (lanes 1,11,20).

3.2. Allele frequencies

Figure 2 summarizes the allele frequency distributions at the loci D5S110 (LH1) and D4S139 (pH30) from 450 unrelated individuals (SW-Germany).

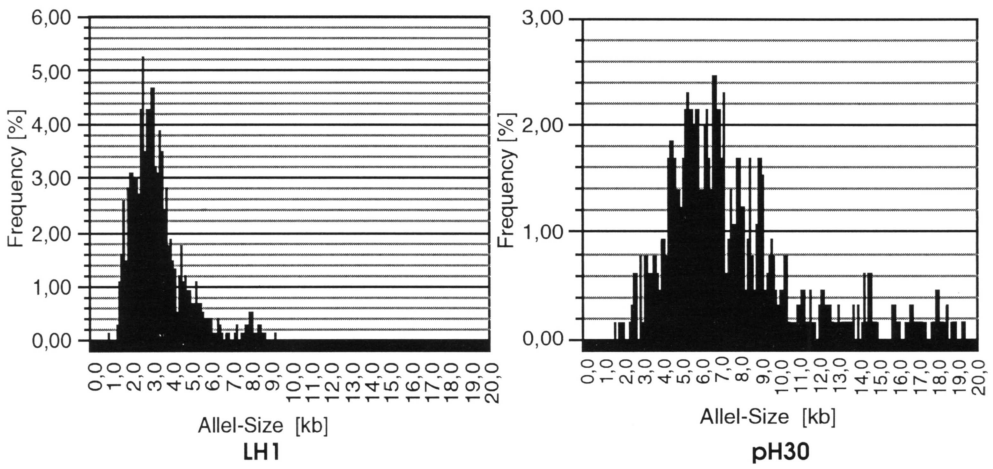

Fig.2: Allele frequency distribution revealed by LH1 and pH30, (n = 450 unrelated individuals)

Major allele clusters were detected by LH1 at 2.6 kb with 5.24%, at 3.1 kb with 4.7% and finally at 2.5 kb and 2.9 kb with 4.3%; pH30 shows 20 peaks, which are broadly distributed between 4 kb and 9 kb with a maximum frequency of 2.45% at 6.7 kb. It is an important and unusual fact that the alleles revealed by pH30 range from 1.5 kb to 19.5 kb with frequencies between 0.002% and 2.5%. The heterozygosity for the locus D5S110 (450 indivduals) and locus D4S139 (450 individuals) was found to be 93.01% and 96.62%, respectively.
The allele frequencies revealed in the small sample of 70 unrelated individuals from N-Portugal were similar to the data determined in SW-Germany, but further studies must be performed to verify these results.

3.3. Mutation rates

We analysed 35 families with 172 children and didn't observe any mutation at the D5S110 locus, but one recombination event at the D4S139 locus, i.e. a recombination rate of 0.0058. Normally the recombination rate will enlarge investigating more families, because it increases with heterozygosity which is at both loci over 93%.

3.4. Statistical evaluations

Table 1 represents the statistical results. We calculated a maximum 3 SD value of 0.0569 or in a percentage deviation of 1.55% for the intergel variation. In comparison to these data the FBI and the Lifecodes Corporation calculated a fixed SD value given as a percentage deviation of 2.5% and 1.8% (Weir, 1992). It is apparent that low measurement errors correspond to precise analysis methods including the experimental, image-processing and calculation procedure.

Table 1: Intergel variation (n=35) in allele sizing of two K562 fragments (f1,f2), detected by LH1 and pH30. AM *arithmetic mean*; SD *standard deviation*; CV *coefficient of variation*.

	LH1		**pH30**	
	f1	*f2*	*f1*	*f2*
AM (kb)	3,691	2,922	6,435	3,424
SD	0,019	0,010	0,028	0,016
CV (%)	0,515	0,338	0,433	0,463
3 CV (%)	1,545	1,014	1,299	1,389

Summarizing all results we conclude that the single-locus probes LH1 and ph30 are valuable complements to the well-estabilised probe-systems like YNH24, EFD52 or TBQ7.

References

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