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Technical note

Genetic polymorphism of the single locus probes pL159-1 and pL355-8

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Summary. The genetic polymorphism of the single-locus probes pL159-1 (D18S17) and pL355-8 (D20S15) was investigated in 445 unrelated individuals using *Pst*I as restriction enzyme. Fragment size calculations were obtained using the molecular weight size marker MW-SBH. The basic relationship between migration distance and molecular weight was transformed using an exponential function. Fragment size frequency data show 2 peaks for pL159-1 at 4.36 kb (2.36%) and 4.67 kb (6.29%) and one peak for pL355-8 at 6.04 kb (5.73%). The rate of heterozygosity exceeded 70% for both probes.

Key words: Single-locus probes pL159-1 (D18S17) and pL355-8 (D20S15) – Fragment size calculation – Population genetic data

Zusammenfassung. Populationsgenetische Untersuchungen der Single-Locus-Sonden pL159-1 (D18S17) und pL355-8 (D20S15) wurden anhand von 445 unverwandten Testpersonen durchgeführt. Als Restriktionsenzym wurde Pst I verwendet. Die Bestimmung der Fragmentgrößen erfolgte mittels des Molecular Weight Size Markes MW-SBH. Die zugrunde liegende Beziehung zwischen Migrationsdistanz und Molekulargewicht wird am ehesten durch eine Exponentialfunktion beschrieben. Erkennbar wird in der statistischen Häufigkeitsverteilung bei der Sonde pL159-1 eine Zweigipfligkeit, die mit 2.36% bei 4.36 kb und mit 6.29% bei 4.67 kb lokalisiert ist, während die Sonde pL355-8 nur einen Peak bei 6.04 mit 5.73% aufweist. Die Heterozygotenrate beider Sonden liegt bei mehr als 70%.

Schlüsselwörter: Single-Locus-Sonden pL159-1 (D18S17) und pL355-8 (D20S15) – Bestimmung von Fragmentgrößen – Populationsgenetische Datenerhebung

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Introduction

Genetically inherited differences based on a variable number of tandem repeats (VNTRs) can be visualized as restriction fragment length polymorphisms (RFLPs). These VNTR loci are informative genetic markers for individual identification (Jeffreys et al. 1985b; Gill et al. 1985; Giusti et al. 1986), parentage testing (Jeffreys et al. 1985a; Baird et al. 1986) and human linkage maps (Nakamura et al. 1988). In recent years different kinds of fragment size calculation methods have been submitted (Southern 1975; Elder et al. 1983; Elder and Southern 1987; Puers and Brinkmann 1991). In this paper we describe a further approach to fragment size calculation and present population genetic data of 2 highly polymorphic VNTR loci, D18S17 and D20S15, detected by the single locus probes pL159-1 and pL355-8.

Materials and methods

Individuals tested. 445 people unrelated by family from the southwest of Germany were analysed.

DNA isolation, restriction, Southern blot. Human genomic DNA was isolated from peripheral blood leucocytes according to the protocol of Miller et al. (1988) and digested with Pst I (Boehringer Mannheim, FRG). The resulting restriction fragments were separated in 0.8% agarose gel at 40 V for 40 h in Tris-borate-EDTA buffer. DNA was transferred by vacuum blotting (Pharmacia-LKB, Freiburg, FRG) onto a nylon membrane (Dianova, Hamburg, FRG), hybridized with the biotinylated probes pL159-1 and pL355-8 (Immucor, Rödermark, FRG) and detected as described in the Dianova manual (Dianova, Hamburg, FRG).

Fragment size calculations. The biotinylated DNA-ladder MW-SBH (Immucor, Rödermark, FRG) with fragment sizes ranging from 23.13 kb–0.17 kb was used as size marker for the standard curve. Three reference lanes (1, 10, 19) are included per gel (20 lanes) from which the standard curve was derived. We have used a digitizing tablet to enter data from sizing membranes onto a com-

puter. Since fragment mobility always varies within one gel, these gel inhomogeneities must first be corrected. We have used a linear interpolation between pairs of the molecular weight marker bands. As a next step the digitized positions of the unknown bands must be converted to molecular weights. For the molecular weight marker used in this study we obtained the best fit using an exponential function $m = a \times e^{(-c \times L)}$ (m = mobility, L = length, a, c = constant factors of each blot characterizing the function computed by at least 2 points). So we calculated the fragment sizes of the unknown band using the transformed formula $\ln m = -c \times L + d + e/L$ (c = inclination, d = axial section, e/L = coefficient of correction).

Statistical evaluation. It is necessary to put forward some statistical parameters to be able to check the precision of a calculation method. Therefore we have used the DNA from one heterozygous person as an internal standard, which was separated on each gel. This way we came up with 30 values for maximum and minimum fragment sizes of each fragment. We have used these data to calculate the arithmetic mean (AM), standard deviation (SD) and coefficient of variation (CV).

Allele frequencies. Allele sizes were stored as arbitrary numbers and presented in a tabulated and graphic form. First we summarized the kb values of the different alleles in a table rounding up to the first decimal point according to the classical mathematical procedure (0.05 kb–0.14 kb = 1 kb) giving "fixed bins" ranging from 0.5 to 0.14 kb units (Table 2). In a second approach the kb-values were rounded to the second decimal point (0.145 kb–0.154 kb = 0.15 kb) resulting in a more detailed overview (Fig. 4).

Results and discussion

VNTR analysis

Figure 1 shows examples of the VNTR patterns from 7 people revealed by the single locus probes pL159-1 and pL355-8 using *Pst*I as restriction enzyme. PL159-1 and

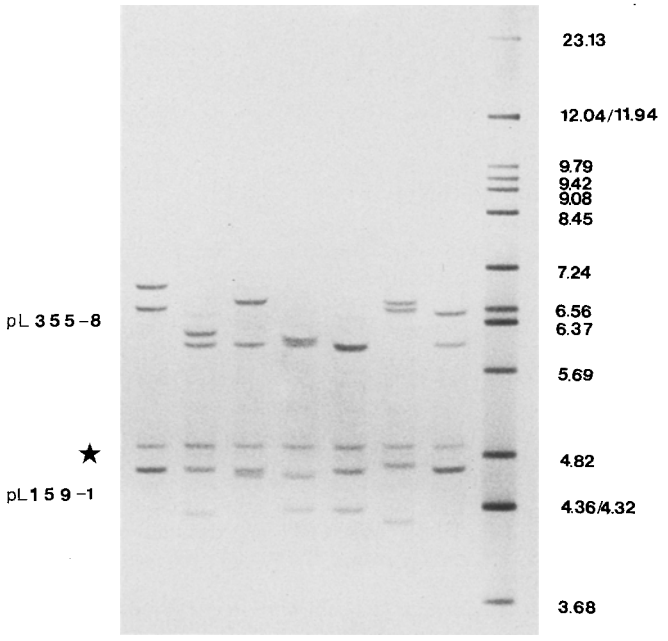

Fig. 1. *Details* of a non-isotopic VNTR analysis with the biotinylated probes pL159-1 and pL355-8, digestion with *Pst*I; molecular weight size marker MV-SBH; *asterisk* = monomorphic band always revealed by pL159-1

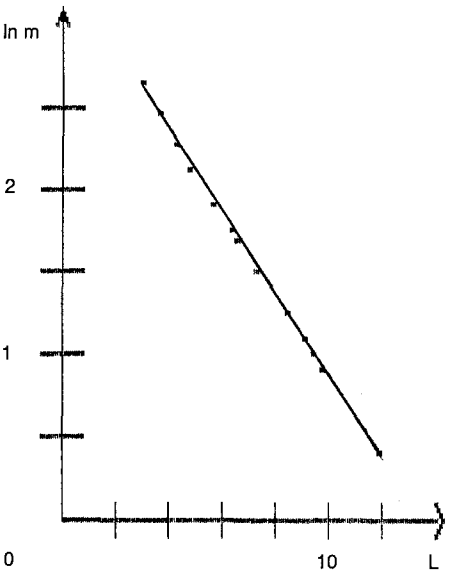

Fig. 2. Diagram of the function $\ln m = -c \times L + d$; *x-axis*: length of the marker fragments (MV-SBH) in kb; *y-axis*: logarithm of mobility in cm of the marker fragment sizes

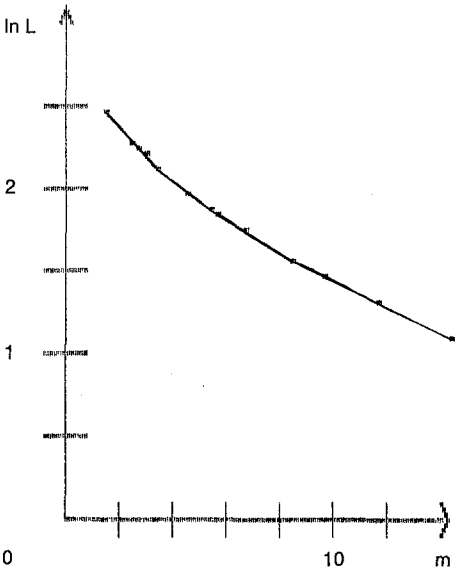

Fig. 3. Diagram of the function $\ln L = a_1 \times m + a_0$ (Fischer and Dingmann 1971); *x-axis*: mobility in cm of the marker fragment sizes; *y-axis*: logarithm of length of the marker fragments (MV-SBH) in kb

pL355-8 detect polymorphic size fragments in the regions 4.0 kb–5.6 kb and 5.7 kb–7.5 kb, respectively. Normally it is possible to combine both probes on one blot, although they detect fragments with similar molecular weights in the kb range 5.6–5.7. PL159-1 always shows an additional monomorphic band marked by an asterisk, which can be regarded as borderline between the polymorphic regions of the 2 probes. From 445 individuals tested we had to repeat 2 sample analyses on separate gels, because it was not possible to definitely assign the banding patterns detected on one gel to one of the 2 probes.

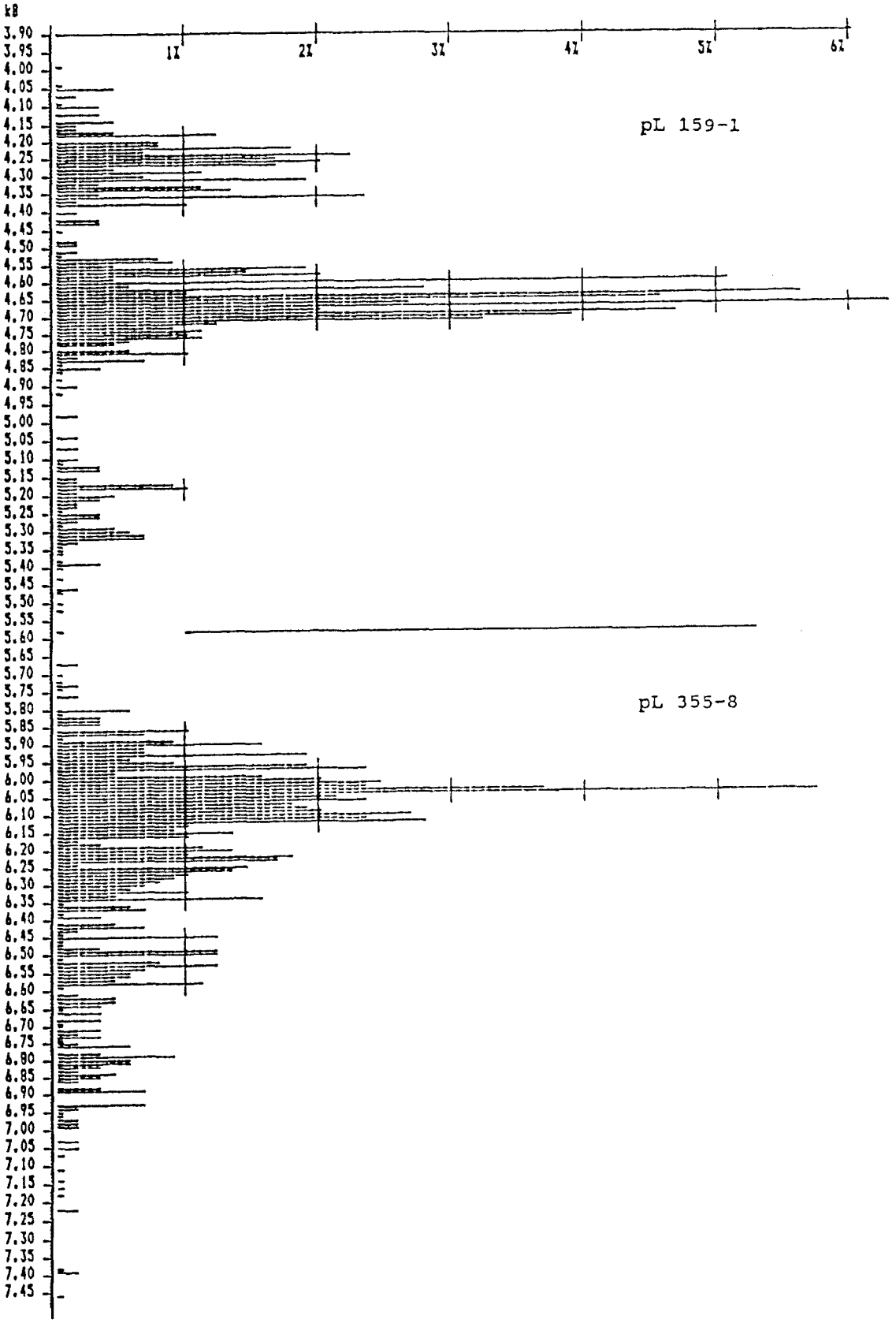

Fig. 4. Allele frequency distribution (%) of 445 unrelated individuals detected by pL159-1 and pL355-8: kb values rounded up to the second decimal point (0.145–0.154 kb = 0.15 kb)

Fragment size calculations

To calculate the fragment sizes of the unknown bands, we have tested several exponential functions, polynomal regression and the reciprocal method. As the molecular weight marker used in this study only shows a few fragments in the region of interest we have decided to use a global fit (least squares) calculating the relationship between length (L) and mobility (m) of the marker DNA fragments according to the following formula $\ln m = -c \times L + d + e/L$ (m = mobility, L = length, c = inclination, d = axial section, e/L = coefficient of correction). Figure 2 shows the graphic representation. A further possibility for a logarithmical analysis $\log L = a_1 \times m + a_0$ (L = length, m = mobility, a_1 = inclination, a_0 = axial section) published by Fischer and Dingmann (1971) is indicated in Fig. 3. The comparison of these 2 methods demonstrates that the formula used by us describes a more linear relationship between L and m. For further investigations concerning the fragment size calculations for the polymorphisms revealed by the probes pL159-1 and pL355-8, we would prefer another molecular weight marker standard. An optimal standard should show 20–30 well-defined and distinctly separated marker fragments, covering the whole range. Using such a standard it is possible to calculate fragment sizes according to the most acceptable local reciprocal method, described by Elder and Southern (1983).

Statistical evaluations

Table 1 shows the statistical results revealed in 30 different runs of one internal standard. We have calculated a standard deviation (SD) with a maximum value of 0.046 for the intergel variation. According to Gill et al. (1990) it might be assumed that 2 bands match if they are within ± 3 SDs of each other. So the true length x of an unknown fragment is enclosed by the interval ± 2.2. These data are in good accordance with the fixed SD value (SD = 2.5), calculated by the Federal Bureau of Investigation (Weir 1992).

Allele frequencies

Table 2 summarizes the different alleles of 445 individuals revealed by pL159-1 and pL355-8. Using the "sliding window" method (Gill et al. 1990) to calculate allele frequencies provides more accurate results but prevents the presentation of all data on a single sheet of paper. Thus we have decided to reduce our sizing data to a frequency table which can be printed on a single sheet and also is compatible with the data of other laboratories. The detected alleles ranged between 3.8–5.6 kb (pL159-1) and 5.7–7.5 kb (pL355-8). Figure 2 presents a more exact overview showing the same fragment size values rounded up to the second decimal point. Two major allele clusters were detected by pL159-1 at 4.36 kb (2.36%) and at 4.67 kb (6.29%). PL355-8 shows only one peak at 6.04 kb (5.73%). The heterozygosity for the locus D18S17 (333 individuals) and locus D20S15 (317 individuals) was found to be 74.8% and 71.5%, respectively.

Table 3 shows the comparison between the frequency data of our study and the data published by Wilting et al. (1992). Minimal differences in the rate of heterozygosity (pL-159-1) and in the kb range of the detected alleles (pL355-8) can be explained by the variable number of

Table 1. Intergel variation (30 repetitive analyses) of an internal standard in allele sizing

	Intergel variation			
Max	6.31	6.11	4.71	4.66
Min	6.18	6.00	4.62	4.57
AM	6.25	6.06	4.67	4.62
SD	0.046	0.043	0.028	0.029
CV	0.74	0.71	0.61	0.63

Max/Min, maximal/minimal fragment size; AM, arithmetic mean; SD, standard deviation; CV, coefficient of variation

Table 2. Allele frequencies of 445 unrelated people revealed by pL159-1 (D18S17) and pL355-8 (D20S15). Kb values were rounded up to the first decimal point (0.05–0.14 kb = 0.1 kb)

Kb	pL159-1			Kb	pL355-8		
	N	Hom	%		*N*	Hom	%
3.8	–	–	–	5.7	7	1	0.8
3.9	–	–	–	5.8	17	3	1.9
4.0	2	0	0.2	5.9	72	14	8.1
4.1	17	2	1.9	6.0	209	50	23.5
4.2	75	6	8.4	6.1	178	31	20.0
4.3	110	7	12.4	6.2	90	11	10.1
4.4	43	3	4.8	6.3	87	4	9.8
4.5	23	4	2.6	6.4	29	2	3.3
4.6	191	31	21.4	6.5	63	7	7.1
4.7	274	50	30.8	6.6	38	3	4.3
4.8	51	7	5.7	6.7	18	1	2.0
4.9	8	0	0.9	6.8	37	1	4.2
5.0	4	0	0.5	6.9	22	0	2.5
5.1	11	0	1.2	7.0	10	0	1.1
5.2	35	0	3.9	7.1	5	0	0.6
5.3	33	1	3.7	7.2	4	0	0.5
5.4	8	1	0.5	7.3	–	–	–
5.5	5	0	0.6	7.4	3	0	0.3
5.6	1	0	0.1	7.5	1	0	0.1

N, Number; Hom, homozygotes

Table 3. Comparison of 2 different studies corresponding to pL159-1 and pL355-8

Probe	*N*	% het	Kb-range	Max %	Kb
pL159-1	445	74.8	4.0–5.6	30.8	4.7
*	209	62	4.0–5.9	34.4	4.7
pL355-8	445	71.2	5.7–7.5	23.5	6.0
*	195	70	5.7–7.9	29.2	6.2

* Wilting et al. (1992)
N, Number; % het, heterozygosity; Max %, maximal frequency data in %, kb-value

people tested. All in all the data revealed in our study are in good accordance with those of Wilting et al. (1992.

These results demonstrate that the polymorphic VNTR Loci D18S17 and D20S15 can be a powerful tool in identity testing, providing the mutation rates have been investigated in large family studies.

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