

# Analysis of forensic stains via PCR amplification of polymorphic simple (gata)<sub>n</sub> repeats

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## INTRODUCTION

Simple repeat sequences which are variable in length serve as highly informative, polymorphic markers in the modern forensic investigation. The typing procedures include multilocus- and single locus probing as well as -nowadays- polymerase chain reaction analysis. Mono-, di-, tri- and tetranucleotide repeats (or "microsatellites") vary extensively in motif numbers and hence in length. After PCR amplification one can demonstrate polymorphic bands in a high resolution polyacrylamide gel. Each allele can be easily sized by comparison with base for base sequenced marker DNAs. More than 150 such microsatellite polymorphisms are described till now (Reis, submitted for publication). In general simple repeats are highly informative given that the number of repeated motifs is higher than 10 (Weber 1990). Because of the high polymorphism information content (PIC) and their technically easy handling simple repeat polymorphisms represent profitable systems for forensic investigations. Under the appropriate precautions PCR can be performed on minute amounts of DNA -even when it is strongly degraded (Roewer et al. 1991, Hagelberg et al.1991). In this report the forensic application of 3 novel tetranucleotide polymorphisms located autosomally and on the Y chromosome is briefly reviewed.

## METHODOLOGY AND RESULTS

We have identified several (gata)<sub>n</sub> simple repeats on cosmid clones 27 and 48 originating from the human chromosomes 12 and the Y. The corresponding plasmid clones 27H39, 4804A and 4815 were chosen for further subcloning and sequence analysis.

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Flanking oligonucleotide primers for identified (gata)<sub>n</sub> stretches at 3 different loci were synthesized. The respective sequences were PCR amplified for 30 cycles in 25  $\mu$ l volumes by the method recommended by Perkin Elmer Cetus. The denaturation temperature was 94°C for 30 sec, primer annealing at 51°C for 30 sec and elongation for 90 sec at 72°C. The conditions are equal for all 3 loci, so that a multilocus PCR is applicable. After LMP gel purification and labeling in a kinase reaction the fragments were separated in a 4% denaturing polyacrylamide gel and exposed to an X-ray film (Roewer et al.1991). In an initial population study (30 caucasians) at each locus a number of different alleles were detected due to variations in the number of gata units. The alleles differ from each other by distinctly 4 base pair repeat units. The alleles are transmitted regularly from father to sons (Y chromosomal locus 27H39LR) and in families (chromosome 12 loci 4804LR and 4815LR)(Fig.1). The heterozygosity rate of the diallelic loci 4804LR and 4815LR is comparable to other single locus systems. Using the observed allele frequencies we calculated the PIC values (according to Botstein et al.1980)(Table 1). The loci are well suitable for forensic investigations because of the shortness of the alleles (132-248 bps), the multilocus PCR approach and the sensitivity. We were able to clarify disputed family relationships by typing of 4 hair roots of each person. The Y chromosomal locus 27H39LR could be used for extremely rapid and sensitive "sexing of stains". Meanwhile the presented (gata)<sub>n</sub> simple repeat loci have been used for actual case work in order to exclude a man on the basis of sperm DNA from suspicion of murder. The amount of extracted DNA from a sperm stain was too low and the DNA was strongly degraded so it was impossible to compare the DNAs by multilocus- or single locus hybridization. We could unequivocally exclude the suspect from causing the stain in all 3 analyzed loci (for details see Roewer and Epplen, submitted for publication).

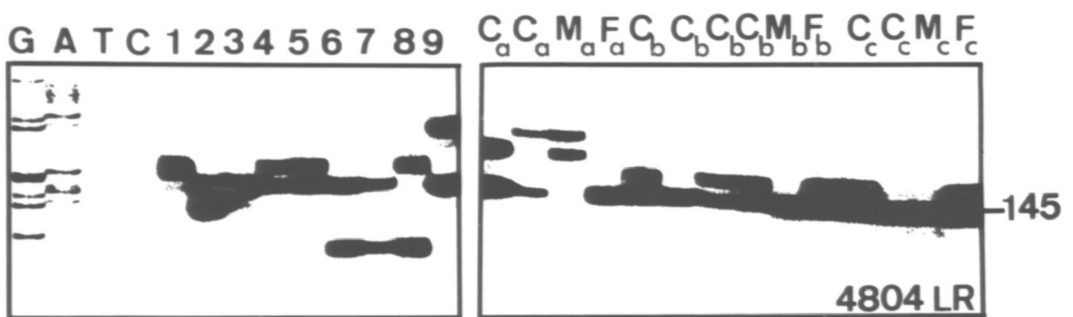


Fig.1. Variability of the simple repeat locus 4804LR as revealed by denaturing polyacrylamide gel electrophoresis. 1-9: unrelated individuals; F: father, M: mother, C: child of family a, b or c. Molecular weight marker (sequenced standard DNA) is shown on the left; the length of one allele, in bp, is depicted on the right

Table 1. Characteristics of autosomal and Y chromosomal, hypervariable simple repeat loci (see also Roewer et al., submitted for publication)

DNA locus (chromosome)	heterozygosity rate (individ.)	PIC	allele frequency (base pair)	pattern of inheritance
4804LR (12)	66.7% (30)	0.61	A(132) 10% B(140) 1.7% C(144) 55% D(148) 20% E(152) 8.3% F(156) 5.0%	codominant (5 fam., 22 meioses)
4815LR (12)	86.2% (29)	0.80	A(222) 3.5% B(226) 1.7% C(230) 6.9% D(234) 29.3% E(238) 19.0% F(242) 20.7% G(246) 8.6% H(250) 10.3%	codominant (5 fam., 20 meioses)
27H39LR (Y)	-	0.65	A(186) 13% B(190) 47% C(194) 23% D(198) 17%	Y chromos. inherited (4 fathers, 4 sons)

#### ACKNOWLEDGEMENTS

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